Brain Activity during Sleep throughout Development in Health and Disease and in the Context of Natural Environmental Influences

Thesis (cumulative thesis)

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by

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'Sleep, that deplorable curtailment of the joy of life,' said Virginia Woolf. Yet Ernest Hemingway loved sleep. 'My life has the tendency to fall apart when I'm awake, you know?'

Table of Contents

Summary

Numerous psychiatric illnesses emerge during childhood and adolescence. Symptom persistence is also higher during this early phase than it is during adulthood. In addition, early onset psychiatric diseases seem to be associated with worse outcomes. Therefore, various strands of research have increasingly focussed on the vulnerable phase of childhood and adolescence. Within this context, sleep research appears to be a promising avenue. Sleep is considered an active process of the central nervous system. Not only does sleep have an impact on processes of cortical plasticity, but it also undergoes major changes throughout development. As a consequence, sleep's relationship to the complex process of maturation in health and disease has recently gained attention.

Building upon these insights, the **first part** of this doctoral project investigated the relationship between sleep and cortical plasticity during development in health and disease. More specifically, the project assessed particular aspects of sleep electroencephalographic (EEG) characteristics in order to better understand healthy and abnormal development. The two main non rapid eye movement (NREM) sleep oscillations – slow waves and sleep spindles – were the main focus of inquiry. Towards this end, the project employed high density EEG, as it offers a unique opportunity to combine temporal with high spatial resolution. Four substantial contributions resulted from this endeavour.

At first, a review article discusses the interplay between sleep EEG characteristics and brain development in health and early-onset psychiatric illnesses. The two subsequent original research articles focus on the most disabling psychiatric illnesses emerging during childhood and adolescence: (1) Schizophrenia and (2) Major Depressive Disorder. This research investigated potential alterations in sleep spindles in adolescents diagnosed with Early Onset Schizophrenia (1), as well as possible modifications in the topography of sleep slow wave activity (SWA) in adolescents diagnosed with Major Depression (2). The project found topographical differences in both disease groups, such as a deficit of spindles in youth with schizophrenia and increased frontal SWA in young patients with depression.

Given that disease patterns can only be understood in relation to our knowledge on healthy individuals, a third article investigates sleep and plastic changes in healthy

development. Therefore, this research studied individual changes in SWA during childhood and adolescence longitudinally, and related possible alterations of SWA to performance in a specific visuo-motor task (3). The data convincingly shows that SWA topography is a trait that can be tracked longitudinally within healthy individuals. Furthermore, the findings suggest that sleep SWA can be used as a mirror for motor skill development and cortical restructuring during healthy adolescence.

Within the first part of the doctoral project, sleep was used as a mapping tool. And yet, an equally relevant question is whether sleep has an active role in mediating cortical plasticity processes. Numerous studies confirmed the beneficial effects of sleep on memory in various tasks. Further, several researchers linked EEG slow waves to sleep dependent memory improvements. Through experimental manipulation, these studies showed that sleep slow waves actively contribute to better performance. To further advance these contributions, in the **second part** of this doctoral project, sleep and learning related plasticity was investigated in the context of natural environmental influences. Thus, healthy young adults were investigated at various altitudes (4, 5). Besides breathing disturbances and slight alterations in sleep structure (4), these results also suggest that slow waves are susceptible to natural environmental influences. Hence, this research proposes that altitude gains ultimately affect sleep dependent performance negatively (5).

In conclusion, this thesis suggested that sleep might be a valuable mapping tool, and that interesting conclusions could be drawn when undertaking external environmental manipulations. Thus, if indeed sleep plays such an active role within numerous processes, investigating the underlying mechanisms of sleep during development in health and disease would truly be an important avenue for future research. As long and short-term plasticity processes seem to collude during adolescence, this appears to be a crucial developmental period to be further studied.

Zusammenfassung

Zahlreiche psychische Erkrankungen entstehen im Kindes- und Jugendalter. Im Vergleich zum Erwachsenenalter ist die Symptompersistenz während diesem Lebensstadium deutlich erhöht. Dazu sind die Langzeitfolgen der frühen Formen psychischer Erkrankungen deutlich konsequenzreicher. Aus diesen Gründen hat sich die Forschung vermehrt mit diesem empfindlichen Stadium der Kindheit und des Jugendalters befasst. In diesem Zusammenhang ist die Schlafforschung ein vielversprechendes Forschungsgebiet. Schlaf wird als ein aktiver Prozess des zentralen Nervensystems betrachtet. Schlaf hat nicht nur einen Einfluss auf Prozesse kortikaler Plastizität, sondern er verändert sich auch erheblich im Laufe der Entwicklung. Folglich hat das Interesse zugenommen, Schlaf im Kontext von Gesundheit und Krankheit während der Entwicklung zu erforschen.

In diesem Kontext hat der **erste Teil** dieser Doktorarbeit den Zusammenhang zwischen Schlaf und kortikaler Plastizität während der Entwicklung untersucht. Genauer wurden spezifische Aspekte von Schlaf elektroenzephalographischen (EEG) Charakteristika erforscht, um normale und abnormale Entwicklungsprozesse besser zu verstehen. Hierbei wurden die zwei wichtigsten NREM-Schlaf Charakteristika – langsame Wellen und Spindeln – mittels hochauflösendem EEG untersucht. Vier Forschungsartikel sind aus diesem Vorhaben entstanden.

Als erstes diskutiert eine Literaturauswertung einen möglichen Zusammenhang zwischen Schlaf EEG Charakteristika während gesunder und gestörter Hirnentwicklung, wobei auf die häufigsten Frühformen psychischer Erkrankungen eingegangen wird. Die folgenden zwei Forschungsartikel befassen sich mit den zwei schwerwiegendsten psychischen Erkrankungen im Kindes- und Jugendalter: (1) Schizophrenie und (2) Depression. In diesem Zusammenhang wurden einerseits mögliche Veränderungen in Schlafspindeln in Jugendlichen mit einer Frühform der Schizophrenie (1), als auch mögliche Abweichungen in der topographischen Verteilung der langsamen Wellen in Jugendlichen mit einer diagnostizierten Erstdepression (2) untersucht.

Um jedoch abweichende Entwicklungsprozesse verstehen und weiterverfolgen zu können, ist das Verständnis von gesunder Entwicklung entscheidend. Aus diesem

Grund wurden in der dritten Forschungsarbeit Schlaf und plastische Veränderungen im Laufe gesunder Entwicklung untersucht. Hier wurden in einer Longitudinalstudie individuelle Veränderungen in der Topographie der langsamen Wellen erforscht und mögliche Veränderungen in der Topographie mit der Leistung in einer spezifischen visuo-motorischen Aufgabe in Zusammenhang gebracht (3). Die Ergebnisse deuten darauf hin, dass die langsamen Wellen im Tiefschlaf auch als Spiegel für motorische Entwicklung – und somit für kortikale Umstrukturierung – genutzt werden können.

Im ersten Teil dieser Doktorarbeit wurde Schlaf vor allem als Abbildungsinstrument verwendet. Jedoch bleibt die wichtige Frage offen, ob Schlaf auch eine aktive Rolle auch in kortikalen Plastizitätsprozessen spielt. Viele Studien haben den förderlichen Einfluss von Schlaf auf Gedächtnisprozesse untersucht. Außerdem haben zeitgemäße Studien die langsamen Wellen im EEG mit schlafabhängigen Gedächtnisverbesserungen in Zusammenhang gebracht. Verschiedene Studien konnten infolge experimenteller Manipulationen vorweisen, dass die langsamen Wellen aktiv zu Leistungsverbesserung führen. Um diesen Forschungsbefunden weiter nachzugehen hat der **zweite Teil** dieser Doktorarbeit sich mit dem Zusammenspiel von Schlaf und lernbedingter Plastizität im Kontext von natürlichen äußeren Einflüssen befasst. Hierbei wurden gesunde junge Männer in unterschiedlichen Höhenlagen untersucht (4,5). Neben Atmungsstörungen und leichten Veränderungen in der Schlafstruktur (4), konnte gezeigt werden, dass Schlaf – hier vor allem die langsamen Wellen im Tiefschlaf – sensitiv auf äußere Einflüsse reagieren (5). Genauer wurde hier gezeigt, dass eine zunehmende Höhenlage schlafabhängige Gedächtnisverbesserungen negativ beeinflusst.

Zusammenfassend konnte gezeigt werden, dass Schlaf als ein wertvolles Abbildungsinstrument dienen kann, und dass weitere interessante Ergebnisse mithilfe externer Manipulation erzielt werden können. Folglich, sollte Schlaf tatsächlich solch eine zentrale Rolle innerhalb mehreren wichtigen Prozessen spielen, dann wäre es von äußerstem Interesse, Schlaf und seine zugrundeliegenden Mechanismen wahrend gesunder aber auch abnormer Entwicklung weiterzuverfolgen. Da kurz- und langzeitige Plastizitätsprozesse während der Entwicklung zusammenzuwirken scheinen, ist es wichtig, diese entscheidende Entwicklungsphase weiterhin zu erforschen.

Research articles included in this doctoral thesis

Research Part 1:

REVIEW Article: Developmental Changes in Sleep and their Relationships to Psychiatric Illnesses

Tesler, N.[1], Gerstenberg, M.[2] and Huber, R.[1]
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Published in: **Current Opinion in Psychiatry** (2013)

Original Research Article (1)
Reduced Sleep Spindle Density in Early Onset Schizophrenia

Tesler, N.[1,4], Gerstenberg, M.[2], Franscini, M.[2], Jenni, O.G.[1], Walitza S.[2], Huber, R.[1,2,3,4]
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Submitted to: **Schizophrenia Research**

Original Research Article (2)
Increased Frontal Sleep Slow Wave Activity in Adolescents with Major Depression and their Unaffected Siblings

Tesler, N.[1,4], Gerstenberg, M.[2], Franscini, M.[2], Jenni, O.G.[1], Walitza S.[2], Huber, R.[1,2,3,4]
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[4] Neuroscience Center Zurich, University of Zurich, Switzerland

Submitted to: **NeuroImage**

Original Research Article (3)
Individual Slow Wave Activity Trajectories as a Marker for Brain Development

Lustenberger, C.[1,2], Mouthon, A-L.[1,2], **Tesler, N.**[1,2], Kurth, S.[1,2,7], Ringli, M.[1,2], Pugin, F.[1,2], Huber, R.[1,2,3,6]

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In Preparation

Research Part 2:

Original Research Article (4)
Are Nocturnal Breathing, Sleep, and Cognitive Performance Impaired at Moderate Altitude (1630-2590m)?

Latshang, T.D.[1], Lo Cascio, C.M.[1], Stöwhas, A-C.[1], Grimm, M.[1], Stadelmann, K.[2,4], **Tesler, N.[3]**, Acherman, P.[2,4], Huber, R.[3,4], Kohler, M.[1,4] and Bloch, K.E.[1,4]

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[4] Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland

Published in: **Sleep** (2013) 36(12): 1969-76F

Original Research Article (5)
Ascent to Moderate Altitude Impairs Overnight Memory Improvements

Tesler, N.[1,2], Latshang, T.D.[4], Lo Cascio, C.M.[4], Stadelmann, K.[3,5], Stoewhas, A-C.[4], Kohler, M.[3,4], Bloch, K.E.[3,4], Achermann, P.[2,3,5], Huber, R.[1,2,3,6]

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Published in: **Physiology and Behavior** (2015) 139: 121-6

1

Introduction & Aims

1.1. General Introduction

Sleep appears to be a general phenomenon, observed across many species, from fruit flies to humans (Siegel, 2008; Tobler, 1995), occupying a significant amount of the lifespan (Cirelli and Tononi, 2008). Taking into consideration that sleep is a state that contravenes reproduction, works against a gathering of nutritional resources, stops social interaction and renders susceptibility to predation, it is perhaps the most puzzling of all organismic behaviours (Abel et al., 2013). Nevertheless, there should have existed a significant evolutionary driving force to opt against such a brain and behavioural state of apparent inactivity. So far, sleep has persisted, suggesting that there must be substantial benefits that compensate for the evident detriments. Moreover, sleep is considered an active process of the central nervous system (Hobson and Pace-Schott, 2002a), being directly involved in cortical plasticity (Diekelmann and Born, 2010; Sejnowski and Destexhe, 2000; Steriade and Timofeev, 2003; Tononi and Cirelli, 2006) and efficient functioning (Tononi and Cirelli, 2006), therefore making it indispensable for a proper development as well as for an efficient adaptation to the alterations occurring in everyday life.

Long before technological and imaging advances shed light on sleep electroencephalographic (EEG) characteristics and their underlying mechanisms, traditional visual stage scoring based on polysomnographic recordings provided insight into the structure of sleep, enabling the major sleep stages to be distinguished (Tesler et al., 2013). During recent years a closer inspection of sleep EEG characteristics proved to be promising to gain insight into processes of brain plasticity. The first part of the introduction will cover aspects of sleep evaluation, starting with qualitative aspects of sleep and subsequently going deeper into more quantitative aspects of sleep, further introducing the two main non-rapid eye movement (NREM) sleep oscillations, slow waves and sleep spindles, both being shown to be involved in cortical plastic processes.

While we are still seeking for the key function of sleep, there is some conformity that sleep serves an important function for the brain. Accordingly, the second part of the introduction elaborates the relationship between sleep and cortical plasticity by highlighting the importance of sleep for memory as well as its contribution to both healthy and disturbed processes of brain function and maturation.

Evaluation of sleep

Compared to wakefulness, brain activity changes dramatically during sleep (Steriade et al., 1993c), showing diverse patterns, further enabling us to differentiate between different sleep stages. The best method to evaluate these alterations in brain activity in both animals and humans is to adopt intracranial or surface EEG. To provide reliability between researchers and clinicians, a standardized scoring manual was released. Up to date, the sleep stage scoring rules, established by Rechtschaffen and Kales in 1968 (Rechtschaffen, 1968) are still used in sleep laboratories. Yet, in 2004, the American Academy of Sleep Medicine commissioned a revision of sleep scoring rules, covering not only sleep stages but also the scoring of arousals or respiratory events, intended for a reliable diagnosis of different sleep disorders. However, after these revisions, the main sleep scoring rules remained similar, despite minor changes in the scoring of deep sleep and further underlining the importance of including electromyography (EMG) and electrooculography (EOG) in addition to the EEG to distinguish between the sleep stages.

Sleep architecture

The sleep EEG is derived from electrodes on the scalp. For the recording of eye movements, electrodes are placed above and below the outer canthus while muscle activity is measured with mental or submental electrodes. Based on frequency, amplitude and waveform of the EEG, sleep can be distinguished from wakefulness and further differentiated into rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep (Figure 1). NREM sleep can be further subdivided into three different sleep stages. During wakefulness alpha activity (8-13 Hz) is prominent, while muscle tone varies in its amplitude and eye movements are apparent. Slow, sinusoidal eye movements indicate the transit to NREM sleep stage 1, where the alpha rhythm is slowly replaced by a low amplitude, predominantly 4-7 Hz activity and sharp vertex waves. Since NREM sleep stage 1 is a transition stage between wake and sleep, after a few minutes spent in sleep stage 1 (N1), individuals typically progress to consolidated stage 2 (N2). The main characteristics of stage 2 are K-complexes and sleep spindles. Thereafter, as sleep deepens, slow waves of low frequency (0.75-4.5 Hz) and an amplitude of >75 µV become evident, being the main feature of deep NREM sleep stage 3 (N3). While in NREM sleep, eye movements are usually barely visible and muscle tone shows rather variable

amplitude, later, irregular, sharply peaked eye movements and a low muscle tone, accompanied by low amplitude, mixed frequency EEG are clear indicators of REM sleep.

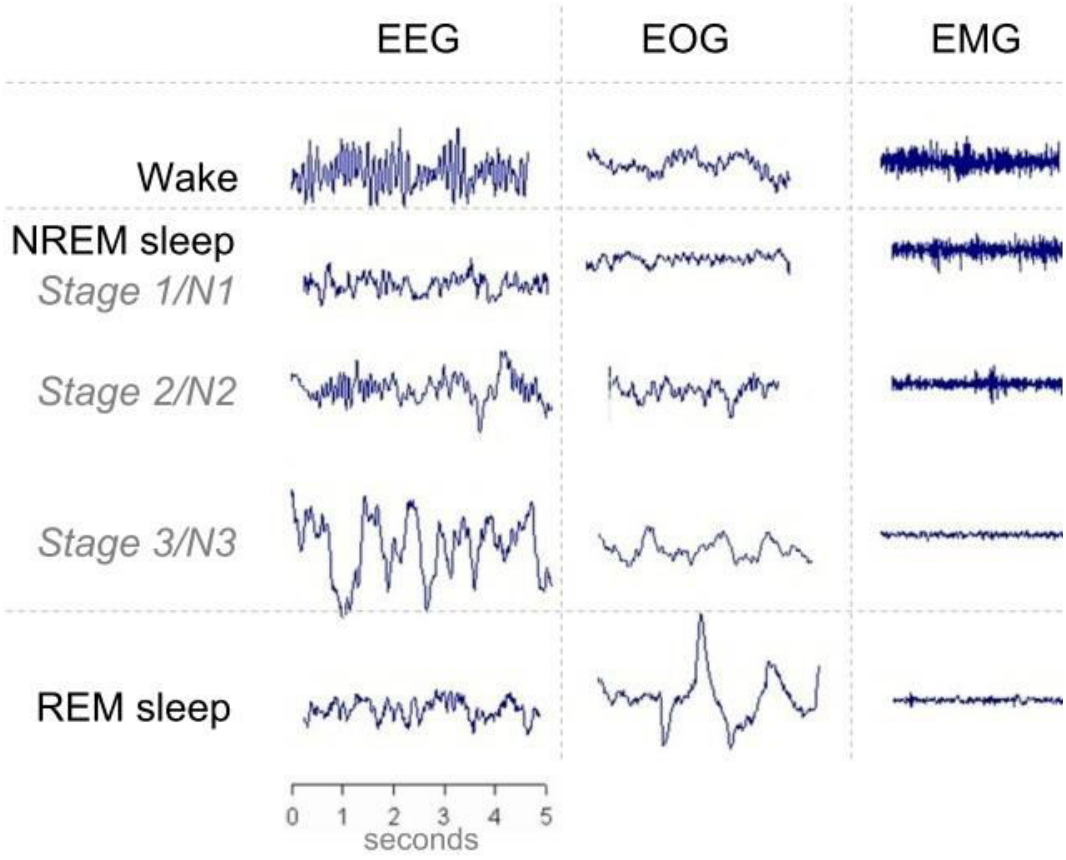

Figure 1: Electroencephalogram (EEG), electromyogram (EMG) and electrooculogram (EOG) recording for different vigilance states. Non-rapid eye movement (NREM) sleep stages N1-N3 are denoted according to (Iber, 2007). This illustration was adapted from Landolt et al. (lecture slides).

A basic feature of sleep is the cyclic alternation of NREM and REM sleep across a period of about 90 to 120 minutes (Feinberg and Floyd, 1979). An eight hour sleep period in a healthy human normally contains 4 to 6 NREM-REM sleep cycles. As illustrated in Figure 2, deep sleep (N3), also termed slow wave sleep (SWS), is most pronounced in the beginning of the night, decreasing thereafter, while REM sleep is rather marginal in the beginning of the night, increasing towards morning hours (Carskadon and Dement, 1994). The sleep architecture derived from the scoring of sleep stages can be visualized in a hypnogram (Figure 2).

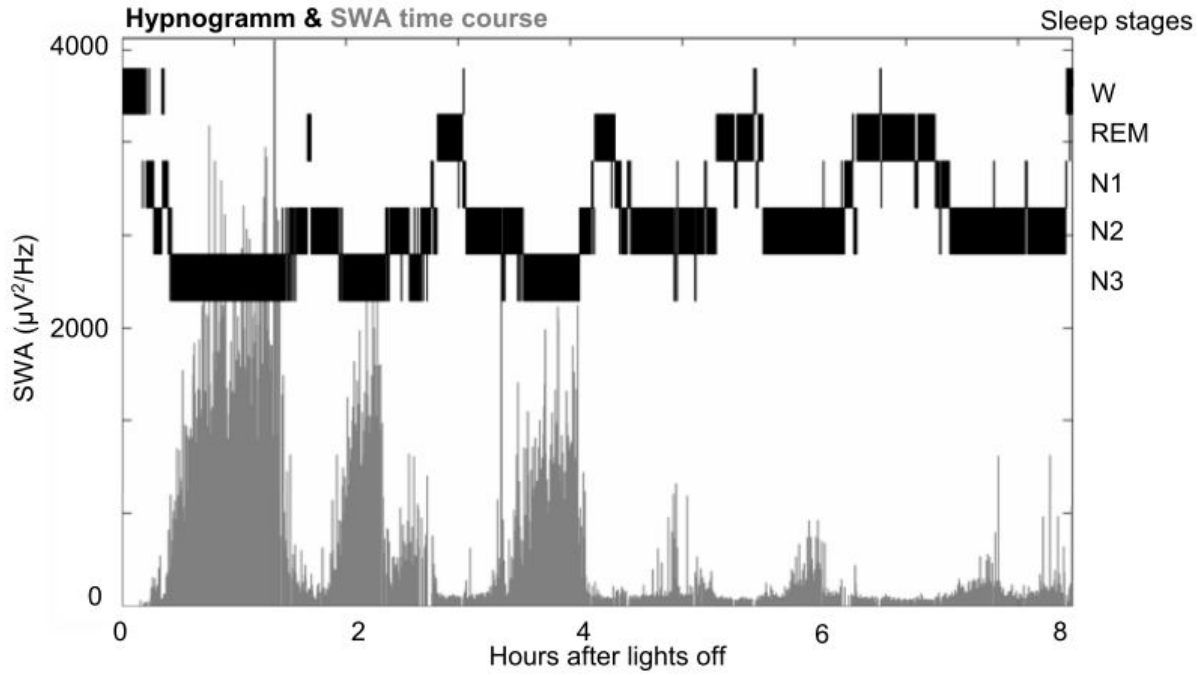

Figure 2: Hypnogram, slow wave activity (SWA) time course and mean power density for an exemplary study night of a 17 year old male subject. The black bars in the illustrate the hypnogram with the different sleep stages; wake, rapid eye movement (REM) sleep; non-rapid eye movement (NREM) sleep stages 1 to 3 (N1, N2 and N3). The SWA (0.75-4.5 Hz) time course is illustrated in grey. This panel has been further cleaned of artefacts.

Spectral analysis

Sleep scoring provides a first evaluation of the sleep structure and consequently about the sleep quality. However, such an evaluation may vary between the scorers (Stanley, 1996). The scoring of the sleep EEG is based on the occurrence of specific brain oscillations of different frequencies. To quantify these oscillations and to achieve a more objective analysis of the sleep EEG, spectral analysis can be performed (Achermann, 2009). Spectral analysis is achieved by Fast Fourier Transformation (FFT) (Cooley and Tukey, 1965). Thereby the time-based EEG is decomposed in frequency components, allowing the analysis of an EEG power spectrum in distinct frequency bands over time and space. The frequency resolution is determined by the length of the analyzed epoch (e.g. 4 s for a resolution of 0.25 Hz). A smoothed power spectrum can be obtained by averaging over several consecutive epochs and frequency bins.

Global and local sleep

Conventionally, the evaluation of sleep by means of sleep stage scoring and spectral analysis is performed with a minimum of 2 EEG electrodes (Rechtschaffen, 1968). However, more recent studies demonstrated that sleep is not a global brain phenomenon but shows topographical variations in different spectral components (Kurth et al., 2010; Lustenberger and Huber, 2012). High density EEG (hdEEG) (with electrode nets up to 256 electrodes) is a method that combines the temporal resolution of EEG with high spatial resolution and therefore allows local sleep components to be displayed (Tesler et al., 2013). Such data is presented by means of colour coded topographical maps, where values are plotted on the planar projection of a scalp model and as a result regional differences become apparent. In the current thesis, hdEEG was used as the method of choice to assess local features of the main NREM sleep oscillations, introduced in the subsequent lines.

NREM sleep oscillations: slow waves and sleep spindles

Slow waves and sleep spindles are two fundamental brain rhythms that are unique to sleep and characteristic for NREM sleep (Figure 3). These two sleep oscillations have been repeatedly linked to learning, memory as well as other processes of brain plasticity, including processes of cortical reorganization. The focus of the current thesis was on these two specific sleep oscillations and their interplay with 1) healthy and disturbed processes of brain restructuring during a vulnerable developmental period and 2) processes of learning and memory, taking into consideration external, environmental influences.

Although the functions of both slow waves and sleep spindles are not yet well understood, several studies have described the mechanisms of their generation and regulation.

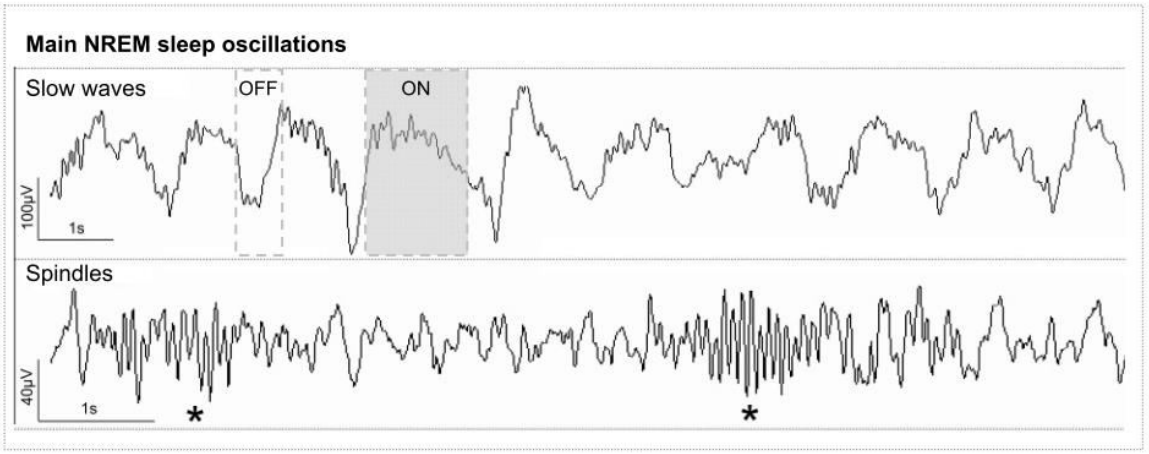

Figure 3: *Top* - example of slow waves during NREM sleep. The highlighted panels exemplify ON and OFF states. *Bottom* - example of sleep spindles during NREM stage 2. Spindles are marked with a star.

Slow waves

Slow waves are characterized as high-amplitude (< 75µV), low frequency (< 5 Hz) waves which predominate during deep NREM sleep (Blake and Gerard, 1937) (Figure 3, top). The activity of sleep slow waves, quantified by 'slow wave activity' (SWA), is a measure representing the spectral EEG power in the low frequency range (0.75 - 4.5Hz) (Achermann, 2009). In the past years a lot of research has been done to better understand its underlying mechanisms.

Origins and mechanisms of slow waves

On the neuronal level, slow (<1 Hz) oscillations are the major electrophysiological feature of deep NREM sleep (Steriade et al., 1993a). When such slow oscillations are synchronized and involve the majority of cortical neurons in a certain brain area, they become visible in the surface EEG as slow waves (Vyazovskiy et al., 2009). Intracellular recordings indicate that sleep slow waves reflect a bistability of cortical neurons undergoing a slow oscillation between two distinct states, each lasting hundreds of milliseconds. Depolarized *up/on states* are episodes of sustained neuronal firing, reflected in the form of a surface-positive wave in the EEG while hyperpolarized *down/off states*, or surface negative waves are episodes of complete neuronal silence (Destexhe and Contreras, 2006; Nir et al., 2011; Timofeev, 2013) (Figure 3, top). It is unique to NREM sleep that virtually every cortical neuron engages in the slow oscillation consisting of alternating periods of sustained

neuronal firing or silence (Amzica and Steriade, 1998; Steriade et al., 1993b; Steriade et al., 2001). Furthermore a close temporal relationship between these cellular phenomena and simultaneously recorded slow waves has been observed (Amzica and Steriade, 1998; Vyazovskiy et al., 2009).

Synaptic strength reflected in SWA

Several recent studies proposed that the high amplitude, low frequency sleep EEG is an indicator of synaptic strength (Hagenauer et al., 2009; Huber et al., 2006; Huber et al., 2004; Riedner et al., 2007; Vyazovskiy et al., 2009; Vyazovskiy et al., 2007). Animal and human studies revealed that synaptic strength, measured as the slope of slow waves, decreases across the night (Riedner et al., 2007; Vyazovskiy et al., 2007). Furthermore, neuronal firing increases during wakefulness and decreases after sleep (Vyazovskiy et al., 2009), suggesting that synaptic strength is upregulated during wakefulness and downregulated during sleep. Researchers developed a model based on findings in animals and humans (Esser et al., 2007, Hill and Tononi, 2005) where they reproduced thalamo-cortical features as well as patterns of neuronal activity during both wakefulness and sleep. The model proposes that high synaptic strength is associated with large amplitude surface slow waves, while low synaptic strength with low amplitude slow waves. Consequently, synchronization is high when connectivity is strong (Esser et al., 2007, Hill and Tononi, 2005). Thereby, during sleep, synaptic strength, along with neuronal synchronization is decreasing, possibly due to a decrease in network connectivity. It has been pointed out that the slope of slow waves represents a good marker of neuronal synchronization: the more synchronized, the steeper are the slopes of slow waves. Consequently, the slope is much steeper at the beginning of the night when sleep pressure is high compared to the slope with the same amplitude towards the end of the sleep period when sleep pressure has dissipated (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007).

Slow wave activity topography

The analysis of the topographical distribution of the most common frequency bands showed that throughout development SWA topography undergoes large changes from childhood to early adulthood, while no such changes can be observed for other frequency ranges (Kurth et al., 2010). Particularly, for different age groups SWA

showed a regional predominance, which shifted from rather posterior brain regions in early childhood to anterior regions in late adolescence and early adulthood (Kurth et al., 2010). These results are in line with findings from other neuroimaging studies, confirming that cortical maturation follows a posterior-anterior time course, with lower order primary areas maturing first, followed by higher order association areas (Gogtay et al., 2004; Sowell et al., 2004). These findings underline the maturational aspect of SWA.

However, next to age-dependent cortical restructuring, mirrored in the topography of SWA, a number of studies illustrated a local regulation of SWA. Local increases of SWA were found after intensive use of specific brain regions (Finelli et al., 2001; Kattler et al., 1994) or after individuals learned a specific task (Huber et al., 2004; Maatta et al., 2010). In particular, visuo-motor learning was associated with a region specific local increase of sleep SWA (Huber et al., 2004). Additionally, a direct link between changes in SWA and altered performance in visuo-motor learning could be shown: a local increase of SWA after visuo-motor learning was positively correlated with improved performance in the following morning (Huber et al., 2004).

Thus, a local increase of SWA seems to parallel processes of long-term refinement during maturation as well as short-term processes of improvement in learning-associated tasks.

Altogether, these experiments indicate that sleep, specifically SWA during deep sleep, may indeed be related to 1. processes of cortical reorganization, with major alterations being observed in the first two decades of life, and 2. use-dependently induced plastic processes in the cortex.

Sleep spindles

Sleep spindles are distinctive EEG oscillations emerging during NREM sleep. They are known as a group of rhythmic waves characterised by progressively increasing, then decreasing, amplitude (Figure 3, bottom). Spindle oscillations appear as brief (0.5 - 3s) episodes of waxing and waning field potentials within a frequency range of approximately 9-15 Hz (Rasch and Born, 2013; Steriade, 2006), though definitions are often variable (De Gennaro and Ferrara, 2003). Spindles are a hallmark for light NREM sleep stages, during which they recur once every 3-10 s together with other

EEG rhythms. However sleep spindles are also found during deeper sleep stages (Rasch and Born, 2013), where they are covered by high amplitude slow waves.

Origins and mechanisms of sleep spindles

Sleep spindles which reside in the thalamic reticular nucleus (TRN) and are under the control of cortical inputs through thalamocortical connections (Kandel and Buzsaki, 1997; Steriade, 2006; Steriade et al., 1993a).

The TRN, the main spindle pacemaker, is located between the thalamus and the cortex and is primarily composed of GABAergic neurons (Guillery and Harting, 2003). It sends inhibitory efferents to all nuclei of the dorsal thalamus while receiving excitatory projections from thalamocortical neurons (Jones, 2007) and inhibitory inputs from the brainstem and basal forebrain (Guillery et al., 1998). Neurons in the TRN possess a specialized congregation of ion channels and mechanisms for intracellular Ca^{2+} handling to maintain the rhythmic burst discharges necessary for spindle generation (Astori and Luthi, 2013). At NREM sleep onset, a progressive hyperpolarization of TRN neurons, caused by glutamatergic input, favours the activation of Ca^{2+} channels that quickly depolarize the membrane voltage and elicits bursts of action potentials (Huguenard, 1996). These bursts are then transferred to cortical neurons, inducing oscillations in the spindle frequency range, which can be detected on the surface EEG as sleep spindles (Steriade and Timofeev, 2003).

Spindle analysis and characteristics

Sleep spindles are defined by standard sleep scoring rules as an activity between 12 and 15 Hz. However, their identification by spectral analysis often does not distinguish between background noise and spindle activity (De Gennaro and Ferrara, 2003). Concentrating solely on the frequency does therefore not allow the detection of other spindle characteristics like spindle amplitude, duration or density (number/minute). The differentiation between these spindle characteristics is important since they may reflect different aspects of sleep spindles. Spindle duration and amplitude are influenced by thalamocortical projections whereas sleep spindle density likely mirrors the activity of the TRN (Bonjean et al., 2011; Fuentealba and Steriade, 2005). Due to the rapidly growing biological interest in sleep spindles and the need to distinguish between different spindle characteristics, next to visual

spindle detection several automated methods of spindle detection were developed to speed up and standardize the process of spindle identification (Warby et al., 2014).

Topography of sleep spindles

Topographical illustration of cortical sleep spindle activity specifies that clear regional variations exist (De Gennaro and Ferrara, 2003). Slow sleep spindles (around 12 Hz) are mostly visible over anterior brain areas whereas fast sleep spindles (around 14 Hz) are more pronounced over centro-temporal and parietal regions (Andrillon et al., 2011; De Gennaro and Ferrara, 2003). Accordingly it was assumed that slow and fast spindles may develop from 2 different cortical sources (Anderer et al., 2001) and may serve diverse functions (De Gennaro and Ferrara, 2003).

High density EEG

The use of high density EEG (with electrode nets up to 256 electrodes) (Figure 4), allows local differences of both SWA as well as sleep spindles to be displayed.

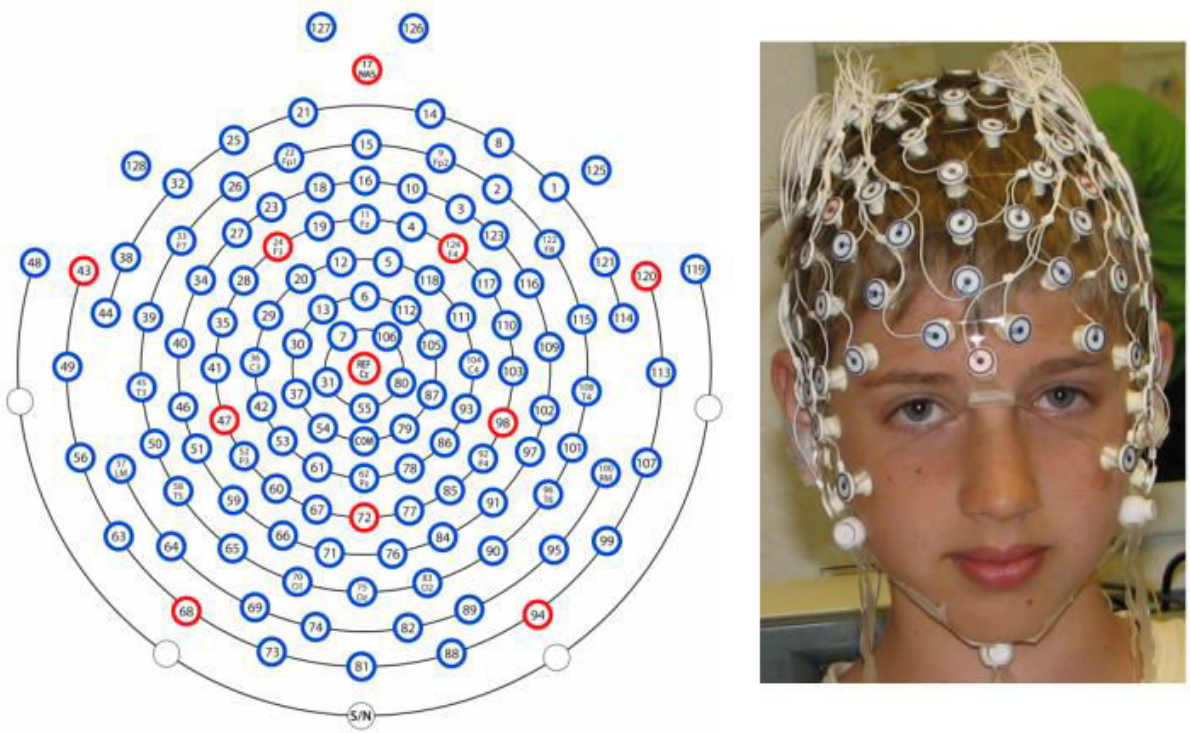

Figure 4: 128 electrode channel map. The right picture shows an 11-year-old boy wearing a high density HydroCel™ Geodesic Sensor Net.

This method provides several advantages compared to several other neuroimaging methods like for example MRI or PET. Due to the high spatial resolution it directly and locally reflects variations in the underlying spontaneous neuronal activity and may therefore be a more direct predictor of functional differences than brain anatomy (Steriade et al., 2001; Timofeev et al., 2001; Vyazovskiy et al., 2009). Furthermore, the assessment of changes in neuronal activity during sleep minimizes possible confounding factors related to waking activities, including changes in the level of attention and distractibility. The perceptual detachment from the environment during sleep might therefore be a further advantage. Moreover, the amount of EEG data that can be gathered during daytime is often limited, whereas data collection during sleep permits the monitoring of hours of brain activity resulting in an easily applicable assessment. The fact that both SWA topography (Figure 5) as well as sleep spindle measures are highly reproducible intraindividually across nights further illustrates the consistency of this method (Lustenberger and Huber, 2012; Tesler et al., 2013).

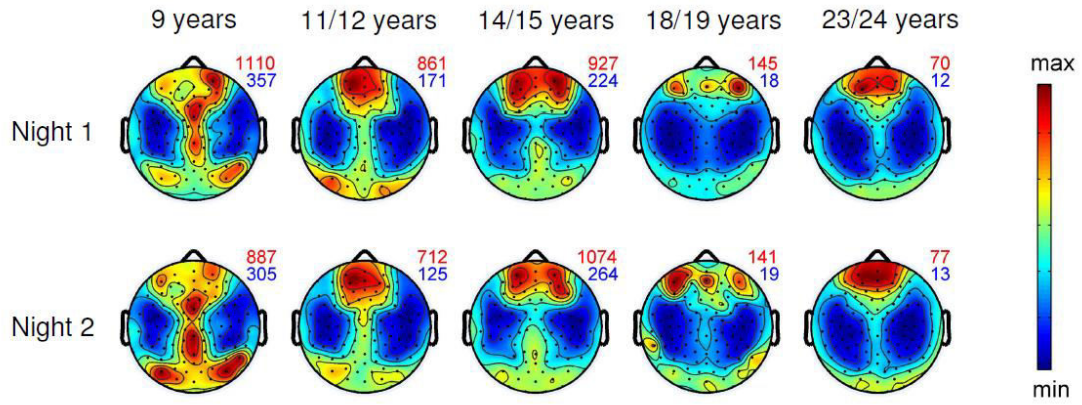

Figure 5: Topographical distribution of slow wave activity (EEG power between 0.75-4.5 Hz) during the first hour of NREM sleep for individuals within defined age-groups. Each column illustrates the SWA distribution of two different sleep sessions (at least one week apart) for one subject. The two 'maps' of each subject illustrate the topographical 'fingerprint' of the power distribution in the SWA range. This illustration was adapted from (Lustenberger and Huber, 2012).

Sleep regulation

The *Two Process Model* (Borbely, 1982) illustrates the importance of two main processes accounting for sleep regulation in humans and in a variety of other species (Tobler, 2000). It is one of the best established models of sleep regulation, proposing a sleep-dependent homeostatic (S) and sleep-independent circadian (C) process to be involved in sleep regulation (Achermann and Borbely, 2011; Borbely, 1982; Czeisler and Dijk, 2001; Daan et al., 1984) (Figure 6).

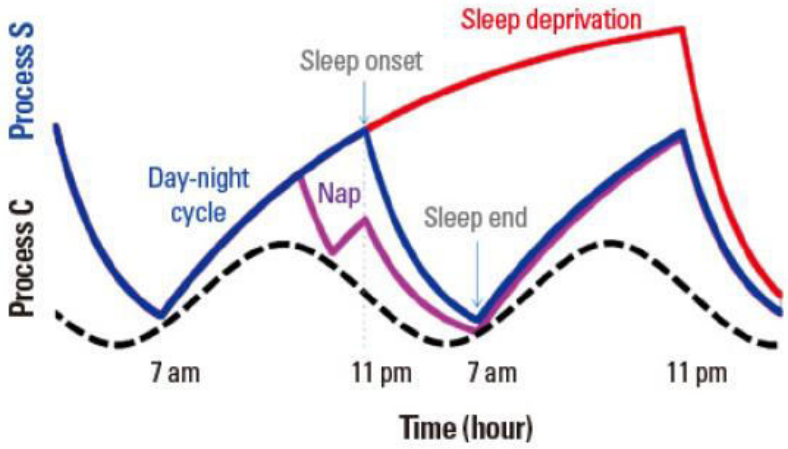

Figure 6: Two Process Model of sleep regulation: the homeostatic process S, in blue and its alterations by sleep deprivation, in red, or by daytime naps, in purple. The circadian process C is illustrated in black. This illustration was adapted from (Borbely, 1982).

Process S reflects the homeostatic aspect of sleep regulation and the need for sleep (Achermann and Borbely, 2011). During wakefulness, sleep pressure along with the need for sleep are enhanced, decreasing thereafter during subsequent sleep. Process S is best reflected by SWA, which has been established as a reliable indicator of sleep depth (Achermann and Borbely, 2011). In 1937, Blake and Gerard (Blake and Gerard, 1937) reported that slow waves in the EEG decline in the course of the night together with a reduction in the arousal threshold. Later on, other studies showed that SWA is highest in the beginning of the night, when sleep pressure peaks, declining thereafter over the course of the sleep period, when sleep pressure is fading (Achermann and Borbely, 2011). Furthermore, it was noticed that SWA was affected by the duration of prior wakefulness. Sleep deprivation studies showed a substantial increase in SWA even though sleep duration is usually unchanged

(Achermann and Borbely, 2011) while daytime naps seemed to decrease SWA and therefore sleep pressure (Figure 6).

The circadian process C is generated by an intrinsic pacemaker, the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus. This master circadian clock controls the circadian rhythm through input via photoreceptive ganglion cells and the retinohypothalamic tract (Klein et al., 1991). Thus, the SCN ensures a proper entrainment of internal rhythms to the daily light-dark cycle as well as to a number of social and environmental Zeitgebers, determining the distribution of sleep over 24 hours.

Even though the *Two Process Model* proposes an independence of the two processes S and C, timing and duration of sleep are described as an interaction of both.

Sleep and plasticity

After the well accepted *Two Process Model,* that assumed the amount of slow wave sleep to be positively correlated with the duration of prior waking (Borbely, 1982; Webb and Agnew, 1971), evidence kept growing that slow waves additionally reflect plastic processes in the brain.

Plasticity might be considered in terms of structure or function. Structural synaptic plasticity within a neural network is responsible for the alterations in the central nervous system (CNS), further reflecting processes of network reorganization (Teyler et al., 1995), while functional plasticity reflects emerging competencies and skill acquisition, best traceable on a behavioural level (Greenough et al., 1993).

Next, I will emphasize the importance of sleep for processes of both structural as well as functional plasticity processes. Since major neurobiological and thus structural alterations occur during the first years of life I will first focus on the relationship between sleep and structural plasticity throughout development. As Lieb (Lieb, 1999) stated, learning happens in each individual throughout life. Consequently functional plasticity is a process that persists even after structural

alterations reached a level of maturation. Therefore I will further stress the importance of sleep for both short and long-term plasticity processes.

Sleep as a contributor to long-term structural alterations throughout development

Sleep structure changes markedly across development with a substantial decline in total sleep time and marked alterations in its composition. In infancy, when the proportion of time awake is smaller than in any other period of life, a large percentage of sleep is spent in REM sleep. Later, when the developing individual spends prolonged intervals awake, the percentage of REM sleep diminishes, whereas the percentage of NREM sleep increases (Roffwarg et al., 1966). Slow waves, during NREM sleep, seem to be related to processes of brain maturation (Kurth et al., 2010). The amplitude of slow waves increases during childhood and is highest shortly before puberty (Feinberg and Floyd, 1979). During adolescence, SWA declines by over 60% between 11 and 16 years, followed by a slowing down of the decline at about 17 years (Feinberg and Campbell, 2010, 2013). As this marked change in SWA is correlated with age, but not with other biological markers of development such as BMI or sexual maturation, (Feinberg et al., 2006), it was proposed that the mechanisms of brain maturation induce those drastic changes in SWA.

However, to move one step away from the surface EEG level and go deeper, age-related changes are detectable also on the neuronal level. During the first years of life neurons establish significantly more connections to other cells (DeFelipe, 1997) while axons explore wider areas on the way to their final target (Gao et al., 1999). Throughout development, more synapses are eliminated than formed (Zuo et al., 2005). Moreover, this synapse elimination, also called pruning, is accompanied by a reorganization of neuronal connections, where unused synapses are eliminated leading to more precise connections. The decline of synaptic density throughout development is also reflected in grey matter changes (Paus, 2005). These changes are in line with the maturation of specific skills (Luna and Sweeney, 2004; Shaw et al., 2006).

Alterations in synaptic density are paralleled by changes in slow wave amplitude (Feinberg, 1982; Huttenlocher, 1979; Huttenlocher and Dabholkar, 1997) and brain metabolism, presumably due to increased energy requirements associated with increased synaptic activity (Chugani, 1998). Several studies support the assumption that sleep SWA is a reliable indicator of net changes in average synaptic density in the course of development (Feinberg, 1982, Huttenlocher, 1979). Since synaptic density can just be determined postmortem in humans, studies started to focus on other neuroimaging methods to be able to approach this issue. Several neuroimaging studies reported early grey matter volume increases and subsequent decreases across a wide area of the cortex throughout development, revealing regional differences in maturation, with some areas maturing earlier than others (Giedd et al., 1999; Giedd and Rapoport, 2010; Gogtay et al., 2004; Sowell et al., 2004).

As the development of the nervous system continues after birth into adulthood (Lenroot and Giedd, 2006; Sturman and Moghaddam, 2011; Tau and Peterson, 2010), so does SWA, paralleling major changes in cortical maturation (Campbell and Feinberg, 2009; Feinberg, 1982; Huttenlocher and Dabholkar, 1997). Furthermore, it is important to mention, that the major changes were restricted to the slow wave frequency range while no such modifications could be observed in other frequency bands (Campbell and Feinberg, 2009; Kurth et al., 2010).

To sum up, the parallel time course of structural reorganizations and SWA indicates that plastic changes throughout development are related to changes in sleep SWA.

Sleep as a contributor to short-term functional plasticity

Evidence for a link between SWA and plastic changes not only arises from studies investigating developmental and thus structural changes, but also from work where synaptic changes were triggered experimentally. Synaptic potentiation by means of transcranial magnetic stimulation (TMS) or by simply performing a particular task and thus enhancing the activity of task-specific brain regions, led in adults to a local increase of SWA over corresponding brain regions (Huber et al., 2007, Huber et al., 2004). Also reduced activity in sensorimotor areas, achieved through arm

immobilization, appeared to produce a local reduction of SWA (Huber et al., 2006). Likewise, in an animal model, deprivation from light after birth resulted in a reduction of SWA restricted to the area of the visual cortex (Miyamoto et al., 2003). Protocols artificially manipulating the level of SWA have shown that slow wave deprivation by acoustic stimuli hindered the performance gains of visuo-motor learning (Landsness et al., 2009). In contrast, artificial boosting of slow waves - by means of transcranially applied oscillating currents - was associated with performance improvement in the declarative memory system (Marshall et al., 2006). These findings support the assumption that SWA plays an active role in the regulation of cortical synaptic strength, thus being further involved in functional plasticity processes beyond structural reorganizational processes occurring throughout development.

Nonetheless, local changes in SWA are also evident after a regular day without any specific experimental manipulation (Cajochen et al., 1999; Finelli et al., 2001; Kurth et al., 2010; Werth et al., 1996), indicating a strong predominance of SWA over frontal regions in adults. These findings highlight functional plasticity processes of SWA, since in adults frontal brain regions are the ones predominantly used (Couyoumdjian et al., 2010; Horne, 1993).

Taken together, there is good evidence that sleep SWA is a reliable indicator of net changes of synaptic strength after intensive/less intensive use of certain brain regions. Consequently, an evident benefit of sleep in this regard are the well observed post-sleep performance improvements (Huber and Born, 2014).

Nonetheless, an interplay of both structural and functional plasticity processes can not be excluded. This statement is relevant for studies focusing on children and adolescents. At this age, the dynamics of structural and functional plasticity seem to peak and both processes can possibly affect each other as well as a proper development.

Sleep as a contributor to memory consolidation

The assumption that sleep contributes to memory consolidation dates years back (Jenkins and Dallenbach, 1924). Starting from there, numerous studies confirmed a beneficial effect of sleep on memory across different learning paradigms (Diekelmann and Born, 2010; Marshall and Born, 2007; Rasch and Born, 2013). Study participants performed better in different tasks after sleep compared to a wake interval of equal length (Fischer et al., 2002; Tucker et al., 2006; Walker et al., 2002). However, there is not just one type of sleep but different sleep stages and sleep oscillations contributing to the tremendous complexity of research investigating the relationship between sleep and memory (Stickgold, 2013). Findings about a relationship between REM sleep and memory consolidation in humans are inconsistent (Rasch and Born, 2013). At present, more evidence exists that suggests the contribution of NREM sleep in processes of memory consolidation. However, not sleep stages itself facilitate memory consolidation, it is rather their underlying electrophysiological characteristics that mediate such sleep-dependent memory formation. Both slow waves and sleep spindles, the two major NREM sleep oscillations, have been related to the beneficial effects of sleep on memory (Rasch and Born, 2013).

Synaptic downscaling and potentiation during sleep

Already in 1949, Donald Hebb (Hebb, 1949) proposed a close relationship between learning and long-lasting local changes in learning-specific networks. He further proposed that recurrent activation of pre- and postsynaptic neurons changes their synaptic properties. Subsequently, several studies confirmed long-lasting modifications due to neuronal activity at almost every excitatory synapse, summarised by the term synaptic plasticity (Malenka and Bear, 2004). The mechanisms underlying synaptic plasticity is long-term potentiation (LTP), leading to an increased postsynaptic response and therefore increased synaptic strength. In contrast long-term depression (LTD) is characterised by a reduction in synaptic strength (Malenka and Nicoll, 1999). However, beside learning (Bliss and Collingridge, 1993; Ito, 2005), synaptic plasticity processes serve also other functions including brain maturation (Johnson, 2001) and cortical remodelling (Kerr

et al., 2011). Therefore, the next part of the thesis gives a more thorough clarification of the plasticity processes occurring during sleep.

The Synaptic Homeostasis Hypothesis (SHH) proposed by Tononi and Cirelli provides important validation for the close relationship between neuronal activity during wake and sleep (Tononi and Cirelli, 2006). Alterations in synaptic strength by means of LTP processes are thought to be the underlying mechanism of learning (Bliss and Lomo, 1973; Whitlock et al., 2006). Learning is a process that does not reach ceiling levels all through life. However, to prevent a saturation of overall synaptic strength due to constant input from the environment as well as to ensure a rather stable learning capacity over years, synaptic strength needs to be rebalanced. Two studies suggested that synaptic strength is homeostatically regulated to promote stability of the system (Turrigiano, 2012; Turrigiano, 1999). The SHH proposes a fundamental role for sleep in this process of synaptic rebalance. The basic idea of SHH rests on the well established homeostatic regulation of sleep, reflected in SWA during NREM sleep (Achermann and Borbely, 2011). The hypothesis integrates three core notions, also illustrated in Figure 7: 1) net synaptic weight increases during wakefulness (synaptic potentiation), 2) SWA reflects the degree of synaptic strength and 3) SWA is associated with a net decrease in synaptic strength (synaptic downscaling). In the following lines these three core notions will be introduced.

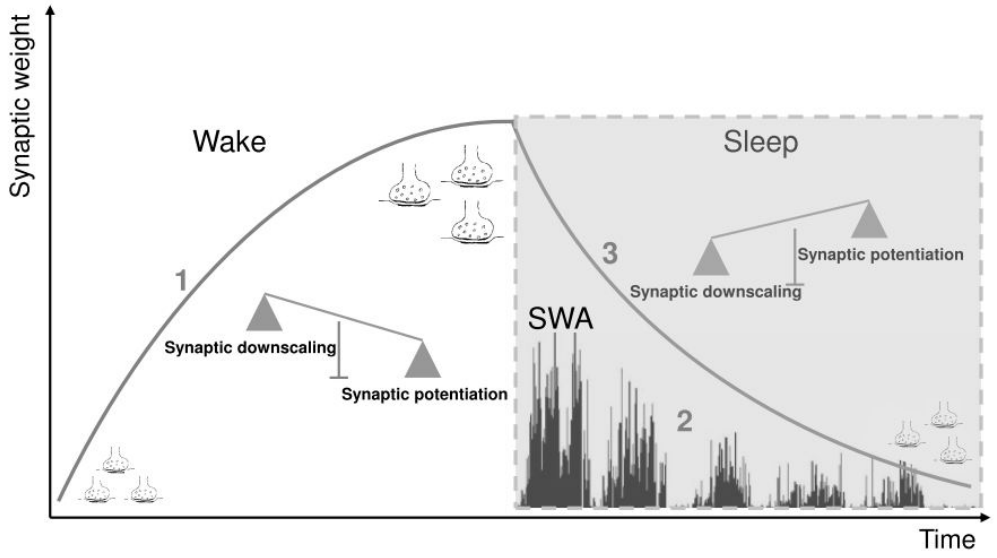

Figure 7: Schematic illustration of the synaptic homeostasis hypothesis (SHH; (Tononi and Cirelli, 2006). Wakefulness is associated with a net increase in synaptic strength, whereas sleep is related to synaptic downscaling, reflected in a decline in SWA in the course of the night. The numbers (1-3) illustrate the three core notions of the hypothesis, explained thoroughly in the text.

1) The net increase in synaptic weight during wakefulness as a consequence of constant input from the environment and learning, has been supported by molecular (Vyazovskiy et al., 2008), electrophysiological (Huber et al., 2013; Liu et al., 2010; Vyazovskiy et al., 2008) and structural evidence (Bushey and Cirelli, 2011; Maret et al., 2011). Interestingly, the more a cortical network undergoes synaptic potentiation (Whitlock et al., 2006), the more SWA is expressed during subsequent sleep. This statement leads to the second core notion of the SHH.

2) The second notion suggests that SWA reflects the degree of synaptic strength. Sleep deprivation studies demonstrated that prolonged wakefulness showed an increased expression of markers for synaptic potentiation along with increased SWA in the following sleep period (Cirelli and Tononi, 2000). Furthermore, in rodents the slope and amplitude of cortical evoked potentials during wakefulness, markers for synaptic strength, were linked to an increase in SWA during subsequent sleep (Vyazovskiy et al., 2008; Vyazovskiy et al., 2011). However, these results were not supported by studies using animal models with where neuradrenergic pathways have been lesioned, leading to reduced expression of potentiation related molecules. These results suggest, that SWA is not related to the period of wakefulness per se, but rather to the amount of synaptic potentiation taking place during wakefulness (Cirelli, 2005; Cirelli et al., 2004; Cirelli and Tononi, 2000). To go one step further and analyze the relationship between synaptic potentiation and SWA, Esser et al. (2007) revealed by means of computational modelling that alterations in synaptic strength are sufficient to impinge on the synchronization of neuronal activity leading to alterations in the amplitude and slope of slow waves. Thus, stronger cortico-cortical connections lead to a more pronounced synchronization, visible in the surface EEG as slow waves with higher amplitude and steeper slopes.

3) The decrease in SWA over the course of a sleep period is reflected in a reduction of synaptic strength during sleep, leading to the third core notion of the SHH, which suggests SWA to be associated with a net renormalization/decrease in synaptic strength (synaptic downscaling). Increases in synaptic strength have a price in terms of energy, space and supply requirements, progressively saturating our capacity to learn (Tononi and Cirelli, 2006). However, during subsequent sleep, we become disconnected from the environment (Steriade et al., 1993b), counterbalancing the net increase in synaptic strength occurring during wakefulness with a net decrease

in synaptic strength. As a consequence, energy, space and supply savings are ensured and the ability for new learning is restored. Such synaptic downscaling depends on the strength of the synapses: very strong synaptic connections are downscaled into weaker connections, but maintained, whereas weaker connections may be entirely removed. SWA was shown to reflect synaptic downscaling and may even contribute to it (Tononi and Cirelli, 2006). There is mounting evidence for synaptic downscaling during sleep in both animals and humans. Molecular studies in rodents and drosophila demonstrated a decline in markers for synaptic strength during sleep (Cirelli et al., 2004; Gilestro et al., 2009; Lante et al., 2011). Some structural evidence exists for homeostatic synaptic alterations, like a reduction in synapse size and number of spines after sleep as well as an increase after wake or sleep deprivation (Appelbaum et al., 2010; Bushey and Cirelli, 2011; Donlea et al., 2009; Maret et al., 2011). What might further contribute to a shift from synaptic potentiation during wakefulness to synaptic downscaling during subsequent sleep are the alterations in the neuromodulatory milieu. Compared to wakefulness, during sleep there is a significant decrease in noradrenalin, histamine, acetylcholine and serotonin together with a low brain derived neurotophic factor (BDNF) (Cirelli and Tononi, 2000; Pace-Schott and Hobson, 2002). An alternative mechanism might be the high firing rates occurring during slow oscillation up-states, leading to a reduction in synaptic strength and therefore to an overall downscaling of the system (Turrigiano, 2012; Turrigiano et al., 1998). In rodents, higher firing rates are found after prolonged wakefulness in the beginning of the sleeping period, decreasing thereafter (Vyazovskiy et al., 2009). A recent study further supported these findings by showing that increased firing rates induced by 0.75 Hz slow oscillatory transcranial direct current stimulation accelerated synaptic downscaling (Reato et al., 2013).

Taken together, many studies support the downscaling mechanism during sleep, proposed by the SHH, however, there are still many unanswered questions, pointing towards a consideration of additional plasticity processes possibly occurring during sleep (Chauvette et al., 2011; Frank, 2012). Contrary to the above mentioned findings, there is increasing evidence that some neuromodulators during sleep favour synaptic potentiation and are therefore increased during NREM sleep (Diekelmann and Born, 2010; Eschenko et al., 2012; Eschenko and Sara, 2008),

suggesting that in addition to global synaptic downscaling also synaptic potentiation processes may occur. In this regard, the consolidation hypothesis proposes that neuronal networks involved in specific tasks during the day are reactivated or 'replayed' during subsequent sleep, leading to a strengthening or potentiation of memory traces (Born et al., 2006; Stickgold, 2005). While during deep sleep or even immobility an interaction with the environment is kept at a minimum level, the hippocampal stratum radiatum maintains irregular large amplitude local field potentials, i.e. sharp waves (Buzsaki et al., 1983; Suzuki and Smith, 1987). These sharp waves are associated with ripples of about 140-200 Hz oscillations in the pyramidal cell layer (Buzsaki et al., 1992; Csicsvari et al., 2000; Ylinen et al., 1995). It has been claimed that a sharp wave ripple can be triggered by phasic inputs, such as slow oscillations or sleep spindles (Clemens et al., 2011; Isomura et al., 2006; Molle et al., 2009; Molle et al., 2006; Sirota et al., 2003; Sullivan et al., 2011). An essential role for sharp wave ripples has been proposed in the information transfer from the hippocampus to the neocortex, associated with a reactivation and thus strengthening of memory traces (Buzsaki, 1989; Eschenko and Sara, 2008; McClelland et al., 1995). On the contrary, also memory deletion was postulated to result from this mechanism (Lubenov and Siapas, 2008). Other research groups considered ocular dominance plasticity in the visual cortex of cats as a proof for synaptic potentiation occurring during sleep (Aton et al., 2009; Frank et al., 2001). In these experiments deprivation of one eye resulted in a rewiring of the visual cortex, favouring the non-deprived eye. NREM sleep served a key function in potentiating cortical responses in the non-deprived eye (Frank et al., 2001).

Even though, at a first glance, synaptic potentiation and synaptic downscaling seem to be two diverse processes that conflict with each other, this is not the case. Wang and colleagues (Wang et al., 2011) consider that local synaptic potentiation may prevent important neuronal networks from global downscaling and weakening while during wakefulness, both synaptic potentiation and downscaling coexist, being crucial for the formation of functional memory traces.

Interplay between the Synaptic Homeostasis Hypothesis and development

The human cortex undergoes striking maturational changes, including alterations at synapse level (Glantz et al., 2007; Huttenlocher, 1979), at molecular level (Lidow et al., 1991), of the dopaminergic system (Weickert et al., 2007), in neuronal growth, in cell death (Levitt, 2003; Von Economo, 1929) as well as in myelination (Deoni et al., 2011; Paus et al., 1999). Neuronal branching and connections to other cells increase during the first years of life (DeFelipe, 1997) and axons explore much wider areas than their final target (Gao et al., 1999). Subsequently, during adolescence, a period of synaptic elimination follows (Zuo et al., 2005). The synaptic elimination during adolescence, termed synaptic pruning, involves a reorganization of neuronal connections, where unused synapses and mistargeted axons are removed, promoting the formation and stabilization of the remaining synaptic connections. Regarding the SHH (Tononi and Cirelli, 2006), slow waves might downscale synaptic connections during the adolescent period in a maturation-specific manner. In this regard, synaptic downscaling would not only reduce synapses until the baseline level is reached but would rather delete even more synapses resulting in a net synaptic decrease (Ringli and Huber, 2011). Consequently, sleep slow waves seem to have a major impact on cortical connectivity, where the process of pruning appears to be essential in the fine-tuning of neural networks during a sensitive developmental period. Nevertheless, pruning during a crucial developmental period may also unmask eventual synaptic deficits (Hoffman and Mcglashan, 1993). May possibly sleep uncover such synaptic deficits and eventually give us further insight into the development of early onset psychiatric disorders? Therefore, the subsequent review article discusses the interplay between sleep and development during a crucial developmental period - childhood and adolescence - , bringing into focus the emergence of the most common early onset psychiatric disorders and their relationship to sleep.

1.2. Sleep and Early Onset Psychiatric Disorders

REVIEW Article: Developmental Changes in Sleep and their Relationships to Psychiatric Illnesses

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Abstract

Purpose of review

Sleep undergoes major changes during development. Its relationship to the complex process of maturation in health and disease has recently received increased attention. This review aims to highlight recent literature examining the interplay of altered sleep, brain development and emerging psychiatric illnesses in children and adolescents.

Recent findings

Beside a temporal relationship of sleep disturbances preceding the onset of psychiatric illnesses, a bi-directional interaction of altered sleep and symptom severity has increasingly been shown. Sleep architecture shows drastic age-dependent alterations on a structural level during the first two decades of life. However, findings regarding disease-specific patterns have remained inconsistent. On a functional level, recent evidence about sleep EEG characteristics points to a close relationship between slow waves, reflecting the depth of sleep, and cortical plasticity.

Summary

Sleep provides a rich source of information to gain insight into both the healthy and disturbed processes of brain function and maturation. Emerging data suggests that the investigation of slow wave activity (SWA) is a novel and promising tool for monitoring both of these processes. It is important to understand when and how deviations from typical developmental sleep alterations occur in order to improve prevention and early treatment of disorders affecting a substantial number of children and adolescents.

Introduction

Many psychiatric illnesses emerge during childhood and adolescence. Symptom persistence is higher during adolescence than during adulthood, and early-onset of psychiatric diseases seems to be associated with marked and often persistent functional impairment as well as increased mortality (Perlis et al., 2004, Kessler et al., 2012). Therefore, research has increasingly focused on the vulnerable phase of childhood and adolescence (Blakemore and Choudhury, 2006, Paus, 2005): a period where substantial neurobiological and behavioural changes occur. Both the complex maturation of the human brain and its major refinements which take place during this critical stage of neural development are challenged by multiple interactions between genetic, epigenetic and environmental factors (Lenroot and Giedd, 2006). A mismatch between the developmental capacities of the individual and the demands generated by the environment can result in compensatory physiological responses. Some demands may provide a stimulus or opportunity for a successful development. Conversely, other demands may overwhelm the system, causing not only high levels of distress but also mal-adaptive neuronal responses involving the structure and function of the developing brain, which can then result in transient or persistent symptoms and psychiatric illnesses (Compas et al., 1995, Lenroot and Giedd, 2006).

The constant modification and refinement of the human brain is not on hold when asleep – quite the opposite seems to be true: sleep is considered an active process of the central nervous system (Hobson and Pace-Schott, 2002a). There is increasing evidence for a close relationship between slow oscillations (<1Hz), a major electrophysiological feature of deep non-rapid eye movement (NREM) sleep, and cortical plasticity (Diekelmann and Born, 2010, Tononi and Cirelli, 2006).

Long before technological and imaging advances shed light on sleep EEG characteristics and their underlying mechanisms, an interplay between sleep disturbances and psychiatric illnesses was noticed (Coble et al., 1979). Traditional visual stage scoring based on polysomnographic recordings provided insight into the structure of sleep, enabling to distinguish the two major sleep stages, NREM sleep and rapid eye movement (REM) sleep. In addition, endophenotypic model

approaches were proposed, suggesting sleep structure to intermediate between the underlying biology and the more complex clinical phenotype (Merikangas et al., 2010, Palagini et al., 2013, Steiger and Kimura, 2010).

However, results have not been consistent, and age-specific aspects were rarely addressed or accounted for. Research investigating the temporal-relationship between altered sleep and the first onset of psychiatric illnesses is generally lacking. Likewise, little is known about the impact of sleep problems on the course of the disease. In search of a possible etiological relationship, investigating sleep alterations on a deeper level, e.g. by a close inspection of sleep EEG characteristics, seems promising in order to gain further insight into the relationship between brain development and emerging psychiatric diseases in children and adolescents.

In the first part of this qualitative review, we will shortly discuss changes in sleep during healthy development. In the second part we will point out the most relevant data examining the interplay of altered sleep structure and onset and course of the most widespread psychiatric illnesses in children and adolescents. In the third part, we will discuss recent findings investigating the interplay between aberrant sleep EEG characteristics and brain development in health and disease. Finally, we will point out why we consider the measurement of one central feature of the sleep EEG – sleep slow wave activity (SWA) – to be a promising tool for displaying early abnormalities in neurodevelopment.

Developmental changes in sleep

Sleep structure changes markedly across development with a substantial decline in total sleep time and marked alterations in its composition (Roffwarg et al., 1966) (see Fig. 1). In infancy, when the proportion of time awake is smaller than in any other period of life, a large percentage of sleep is spent in REM sleep. Later, when the developing individual spends prolonged intervals awake, the percentage of REM sleep diminishes, while the percentage of NREM sleep increases (Roffwarg et al., 1966) (see Fig. 1). Slow waves are the dominant electroencephalographic (EEG) characteristic of NREM sleep and are most prevalent during deep sleep, so-called slow wave sleep (SWS). The activity and distribution of slow waves that are visible

on the surface EEG have a typical pattern of large waves with high amplitude (>75 µV) and low frequency (<4.5 Hz). Specifically, the fundamental cellular phenomenon underlying EEG slow waves are cortical slow oscillations, reflecting near-synchronous transitions between episodes of neuronal firing and states of complete neuronal silence (Vyazovskiy et al., 2007). The activity of sleep slow waves, quantified by "slow wave activity" (SWA, EEG power between 1 and 4.5 Hz), mirrors the depth of sleep (Borbely and Achermann, 2005) and seems to be related to processes of brain maturation (Kurth et al., 2010b). The amplitude of slow waves increases during childhood and is highest shortly before puberty (Feinberg and Floyd, 1979). During adolescence SWA declines by over 60% between 11 and 16 years followed by a slowing down of the SWA decline at about 17 years (Feinberg and Campbell, 2010b, Feinberg and Campbell, 2013). Since this marked change in SWA is correlated with age, but not with other biological markers, of development such as body mass index, or sexual maturation, Feinberg et al. (Feinberg et al., 2006) hypothesized that mechanisms of brain maturation induce those drastic changes in SWA.

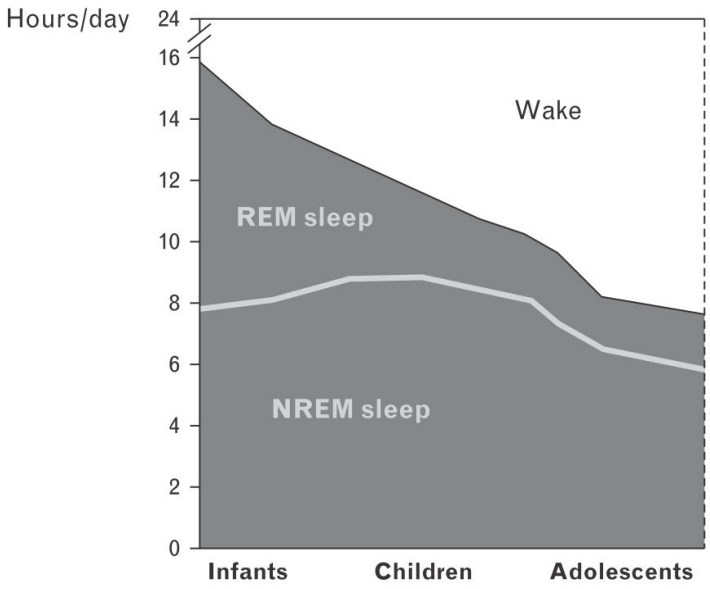

Figure 1: Hours spent in REM sleep, NREM sleep and waking over the first 2 decades of life. NREM, non-rapid eye movement; REM, rapid eye movement. Adapted with permission from Roffwarg et al., 1966.

Sleep structure and its interplay with mental disorders in children and adolescents

Psychiatric illnesses are highly prevalent in children and adolescents with up to 49.5% meeting criteria of at least one diagnostic category in the United States (Kessler et al., 2005). Anxiety disorders can be detected in almost 1 of 3 adolescents, followed by impulse-control disorders in almost 1 out of 5, and mood disorders in almost 1 out of 7 adolescents (Merikangas et al., 2010). The onset of these 3 broad classes of disorders seems to be age-related (Merikangas et al., 2010) (see Fig. 2). Impulse-control disorders such as attention-deficit and hyperactivity disorder (ADHD) occur in early childhood and their incidence increases steadily during adolescence. Anxiety disorders, particularly specific phobias and separation anxiety, emerge during early childhood and their incidence increases steeply until the age of 12. Mood disorders, such as major depressive disorder (MDD) and bipolar disorder (BPD), emerge rarely during childhood, but their incidence shows a nearly two-fold increase from age 13 to 17 (Merikangas et al., 2010) (see Fig. 2).

In line with the mentioned age-specific onset, we will discuss altered sleep, particularly SWS and the most prevalent psychiatric illnesses, separately and, where possible, point out common patterns.

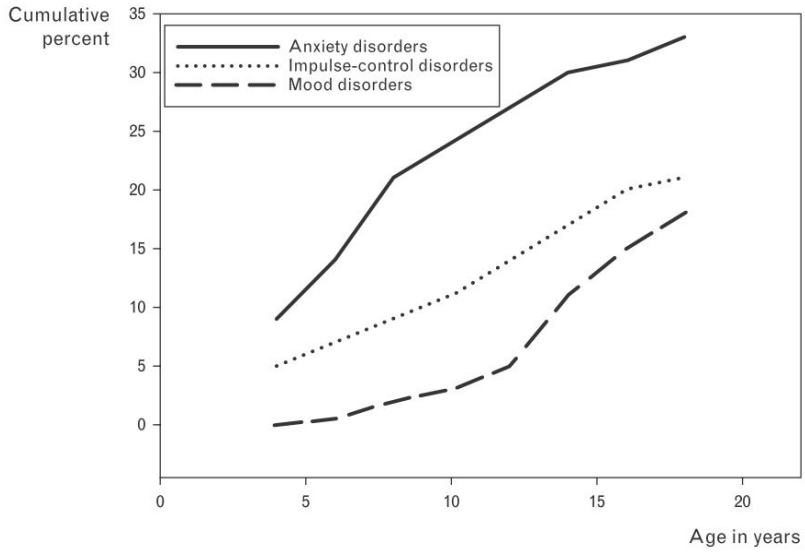

Figure 2: Cumulative lifetime prevalence of major classes of DSM-IV disorders among adolescents (n=10123). Adapted with permission from Merikangas et al., 2010.

Impulse-control disorders

A recent review revealed that 30% of children and 60-80% of adults with ADHD have symptoms of sleep disorders, such as daytime sleepiness, insomnia, restless legs syndrome, and sleep disordered breathing (Yoon et al., 2012).
When examining the structure of sleep in children with ADHD, sleep onset latency was prolonged, the number of stage shifts per hour of sleep was increased and sleep efficiency was lower than in healthy controls (Cortese et al., 2009). Additionally, increased movements during sleep and increased daytime sleepiness were also reported (Cortese et al., 2009, Konofal et al., 2010, Sadeh et al., 2006). However, findings are variable and it was suggested that this variability is mainly due to a highly heterogeneous group of subjects, e.g. including children with all three subtypes of ADHD, the predominately inattentive, the predominately hyperactive, and the combined type (Yoon et al., 2012).
In a recent longitudinal study, Scott et al. (Scott et al., 2013) showed that an age-specific decrease of more than one standard deviation in sleep duration, e.g. between age 3 and 5 was a significant predictor of ADHD. The authors concluded that the intra-individual rate of change in sleep duration, rather than the inter-individual difference of absolute sleep duration, may prove particularly beneficial in identifying an increased risk of ADHD.
Thus, shorter sleep duration and sleep disturbances seem to predate the onset of a full clinical syndrome defining the clinical diagnosis (Scott et al., 2013). Importantly, the association of sleep related problems and ADHD also seems to contribute to a less favourable outcome (Konofal et al., 2010, Spruyt et al., 2012).

Anxiety and mood disorders

Sleep related problems in early childhood seem to precede symptoms of anxiety in adolescence and frank anxiety disorders in adulthood (Gregory et al., 2005, Gregory and O'Connor, 2002, Jansen et al., 2011). Likewise, reported sleep problems seem to precede depressive symptoms as well as the first MDD or BPD episode in adolescents and adults (Gregory et al., 2009, Leopold et al., 2012, Riemann and Voderholzer, 2003). Sleep disturbances were the most prevalent symptoms that patients experienced prior to onset of BPD (Jackson et al., 2003), which caused

early recognition centres to focus on sleep disturbances in their novel risk assessment tools (Forbes et al., 2008, Riemann and Voderholzer, 2003).

When comparing the sleep structure in children and adolescents with anxiety disorders to those with MDD, it was found that only the anxiety group exhibited an altered pattern, such as more awakenings and markedly reduced SWS (Forbes et al., 2008). In contrast, two longitudinal studies reported altered sleep structure, such as reduced REM sleep latency and more REM sleep, to precede and co-occur in adolescents with MDD (Dahl et al., 1996, Rao et al., 2002). SWS seemed to be reduced only in a subgroup of depressed children who later developed a BPD (Rao et al., 2002).

Further insight into both protective and disease-promoting factors was provided by studies in children and adolescents considered to be at high risk for developing a depressive episode due to having a first-degree relative with a history of depression. In a cohort of pre-pubertal at-risk children, those who spent more time in SWS and who had less difficulties falling asleep seemed to be less likely to develop a depressive episode as young adults (Silk et al., 2007).

Interestingly, Bat-Pitault et al. (Bat-Pitault et al., 2013) revealed an age-specific aspect of altered sleep structure. Children at-risk showed no significant structural alterations, whereas at-risk adolescents exhibited increased stage decreased SWS, and shortened REM sleep latency. In adults with MDD, reduced SWS is a quite consistent finding and was shown to be associated with a less favourable outcome of the disease (Ehlers et al., 1996, Hatzinger et al., 2004, Modell et al., 2002, Modell et al., 2005). Moreover, anxious or depressive symptoms and sleep disturbances seem to reinforce each other in a bi-directional manner (Alfano et al., 2007, Hansen et al., 2013). In children and adolescents with MDD, sleep disturbances are not only associated with more severe symptomatology, but also with longer depressive episodes (Liu et al., 2007), increased risk for relapse after remission of depression (47), and a greater risk for suicide ideation (Emslie et al., 2012, Franic et al., 2013, Urrila et al., 2012) and completion (Goldstein et al., 2008). The association between sleep disturbances and suicidal ideation was further underlined by the finding that 86.7% of 11-13 year-old adolescents who reported suicidal ideation also reported sleep problems (Franic et al., 2013). Moreover, sleep disturbances are associated with poorer response to treatment in adolescents diagnosed with MDD (Emslie et al., 2012, Urrila et al., 2012). Interestingly, children with MDD and insomnia are shown

to be more responsive to anti-depressive medication than adolescents (Emslie et al., 2012).
In adolescents diagnosed with BPD, sleep disturbances and structural aberrations, such as shorter total sleep time and greater sleep variability, were correlated positively with severity of manic and depressive symptoms (Gruber et al., 2011, Lunsford-Avery et al., 2012). Follow-up investigations have shown this correlation to be stable (Gruber et al., 2011, Lunsford-Avery et al., 2012).

Taken together, it appears that reported sleep problems are highly prevalent in all of the psychiatric illnesses with paediatric onset discussed above. Sleep problems seem to precede the onset of ADHD, anxiety disorders, MDD and BPD in youth. Researchers addressing early detection and prevention of MDD and BPD consider disturbed sleep as a vulnerability marker possibly predicting the later onset of the disease. Sleep architecture in psychiatric diseases shows alterations with age-dependent aspects and several disease-specific patterns, but results are still inconsistent and data are insufficient to draw further conclusions. In their review of sleep and ADHD, Konofal et al. (Konofal et al., 2010) calls this disease a “24-hour” disorder, stressing that sleep disruptions contribute to daytime symptomatology. As discussed above, this seems to be true for all the mentioned psychiatric illnesses emerging during childhood and adolescence. However, the underlying mechanisms of the interplay of altered sleep and psychiatric illnesses remain unclear. New promising approaches focusing on sleep EEG characteristics discussed below provide insight into the neuronal mechanisms and possible causal interactions.

Sleep EEG characteristics and cortical plasticity

As the development of the nervous system continues after birth into adulthood (Lenroot and Giedd, 2006, Sturman and Moghaddam, 2011, Tau and Peterson, 2010), so does SWA, paralleling major changes in cortical maturation (Campbell and Feinberg, 2009, Feinberg, 1982, Huttenlocher and Dabholkar, 1997). Furthermore, maturation of sleep SWA topography matches the time course of several MR derived markers of brain maturation, e.g., gray matter volume (Buchmann et al., 2011). High density EEG (with electrode nets up to 256 electrodes) is a method that combines the temporal resolution of EEG with high spatial resolution and therefore

allows to display local differences of SWA. It was shown that the location on the scalp over which maximal SWA can be measured proceeds in a 'back to front' direction, being maximal over brain regions maturing at that time (Kurth et al., 2010b) (see Fig. 3, top).

Moreover, several studies have shown a local regulation of SWA. Local increases of SWA were found after intensive use of these specific brain regions (Finelli et al., 2001a, Kattler et al., 1994) or after subjects learned a specific task (Huber et al., 2004, Maatta et al., 2010). More specifically, visuo-motor learning was associated with a region specific local increase of sleep SWA (Huber et al., 2004). In contrast, reduced activity in sensori-motor areas, achieved through arm immobilization, appeared to produce a local reduction of SWA (Huber et al., 2006). Likewise, in an animal model, deprivation from light after birth resulted in a reduction of SWA restricted to the area of the visual cortex (Miyamoto et al., 2003). In addition, a direct link between changes in SWA and altered performance in visuo-motor learning could be shown: a local increase of SWA after visuo-motor learning was positively correlated with improved performance in the subsequent morning (Huber et al., 2004). Protocols artificially manipulating the level of SWA have shown that slow wave deprivation by acoustic stimuli hindered performance gains of visuo-motor learning (Landsness et al., 2009). In contrast, artificial boosting of slow waves – by means of transcranially applied oscillating currents – was associated with performance improvement in the declarative memory system (Marshall et al., 2006).

Taken together, all these experiments indicate that sleep, specifically SWA during deep sleep, may indeed be related to maturational as well as use-dependently induced plastic processes in the cortex.

Sleep EEG characteristics and its interplay with mental disorders

In the context of psychiatric illnesses these changes in SWA have been first brought up in a recent study by Ringli et al. (Ringli et al., 2013), investigating children diagnosed with ADHD. A different SWA distribution in children with ADHD in comparison to healthy children was displayed. The local distribution paralleled

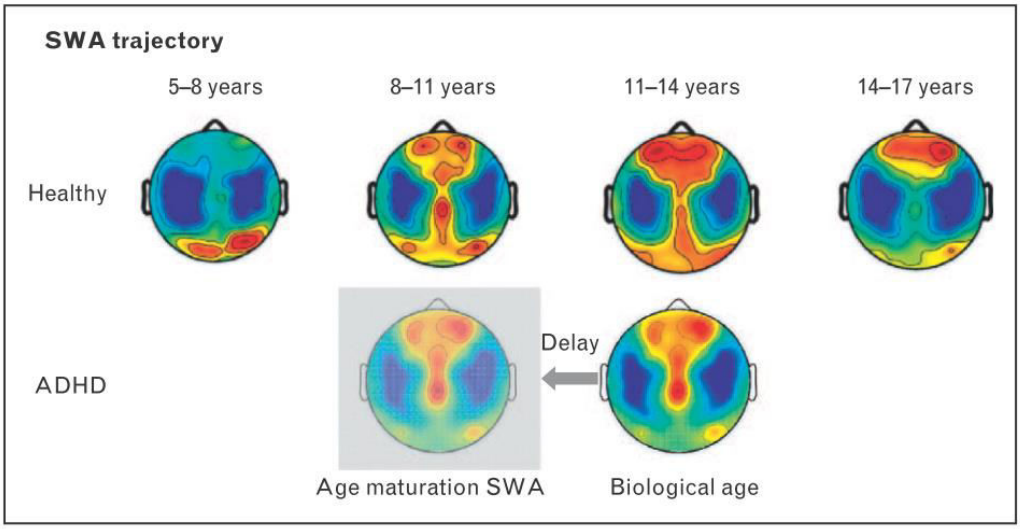

Figure 3: Topographical distribution of SWA for different age groups. Top: healthy population. SWA topography shows a 'back to front' shift from childhood to late adolescence. Bottom: children diagnosed with ADHD. SWA of children with ADHD (mean age 12 years) shows a maturational delay compared with the healthy group. The SWA pattern fits well the SWA distribution of the younger 8-11-year-old healthy group. ADHD, attention-deficit hyperactivity disorder; SWA, slow wave activity. Adapted with permission from Kurth et al., 2010 and Ringli et al., 2013.

the pattern seen in younger children with more pronounced SWA over central brain areas (Kurth et al., 2010b) (see Fig. 3, bottom). This delay in the typical 'back to front' brain maturation is in accordance with findings from neuroimaging and behavioural studies proposing a maturational delay in ADHD (Drechsler et al., 2005, Gustafsson et al., 2010, Kinsbourne, 1973, Shaw et al., 2007). Ringli et al. (Ringli et al., 2013) further hypothesized that the local SWA increase over the motor cortex is linked to increased motor activity during daytime. Hyperactivity is one of the core symptoms in ADHD and, as seen in the above discussed use-dependent changes in SWA, sleep SWA might locally increase in the brain regions that were previously used intensely. Although both the maturational as well as the use-dependent hypotheses are plausible, one additional observation favours the maturational hypothesis: daytime activity, assessed by continued monitoring of motor activity with wrist actigraphy, was not different between the ADHD and the healthy control group (Ringli et al., 2013).

To date, studies investigating sleep EEG changes in children, adolescents or adults diagnosed with an anxiety disorder are missing. With regard to mood disorders, SWA was examined in adults illustrating that young depressed women showed

higher frontal EEG delta activity compared to healthy young and older women (Frey et al., 2012). Furthermore a reduction in depressive symptoms correlated with the overnight dissipation of fronto-central SWA (Landsness et al., 2011). Frey et al. (Frey et al., 2012) hypothesized that the local increase of SWA over frontal brain regions in depressed individuals might be due to increased rumination during daytime, maladaptive repetitive self-focused thinking, a common symptom of depression. This hypothesis is in line with results from a study investigating the neuronal correlates of rumination using functional magnetic resonance imaging, revealing higher activation in the medial and dorsolateral prefrontal cortex and in limbic structures during rumination (Cooney et al., 2010). However, in the study by Frey et al. (Frey et al., 2012) the link between SWA and rumination was not further investigated. Thus, to establish an association with a pathophysiological correlate of the disease requires additional exploration. Finally, in a recent paper by Lopez et al. (Lopez et al., 2012) the accumulation and dissipation of SWA was shown to be abnormal in depressed male adolescents. The authors concluded that SWA abnormalities in adolescents diagnosed with depression may relate to different depressive symptoms in both females and males.

In summary, limited research has been conducted on sleep EEG characteristics in children and adolescents with mental disorders. However, findings from normal development and the first results focusing on SWA topography in ADHD look promising suggesting that SWA may be a valuable tool that can give further insight into the neuronal correlates and possible maturational and use-dependent modifications preceding and/or occurring in the context of psychiatric diseases.

SWA topography as a novel tool

To diminish the enormous burden of early-onset psychiatric diseases, a proper diagnosis and early treatment of emerging psychiatric disorders is needed but this remains challenging at the same time. Diagnostic criteria rely on reported symptoms and symptom expression at this age can be less pronounced or atypical, which might further contribute to the longer lag between the onset of disease and first treatment in children compared to adults (Berk et al., 2011, McGorry et al., 2011). Consequently, there is a strong need to improve the detection of underlying mechanisms and to display early changes. May the topography of SWA respond to

these demands? While other neuroimaging techniques like PET and fMRI offer superior spatial resolution, high density EEG during sleep combines superior temporal resolution with high spatial resolution (Lustenberger and Huber, 2012). Interestingly, the local increase of SWA shows considerable overlap with the brain's "default network", a set of regions characterized by increased neural activity during wakeful rest, detected by fMRI (Dang-Vu et al., 2008, Murphy et al., 2009). Still, resting-state fMRI is susceptible to many confounding factors such as attention, motivation and distractibility. The perceptual disconnection from the environment during sleep might therefore be a further advantage and particularly relevant for studies in children with psychiatric diseases. The fact that SWA topography is highly reproducible intraindividually across nights further illustrates the consistency of this method (Finelli et al., 2001a, Huber et al., 2004, Lustenberger and Huber, 2012). Furthermore the near-synchronous transition between episodes of neuronal firing and states of complete neuronal silence, a typical characteristic of deep sleep (Steriade et al., 1993a) is a further advantage of adopting SWA as a mapping tool, because compared to wakefulness, which displays a blend of activities in different frequency ranges, the healthy pattern of low frequency slow waves occurring repeatedly during sleep has been intensively investigated and is thus well understood. Therefore, a deviation from this typical pattern becomes detectable and potential mechanistic explanations might be feasible. Also, sleep recordings are easily applicable, cost-efficient, non-invasive and can be repeatedly applied. It will be of great interest to assess sleep SWA topography also in a broader range of psychiatric illnesses and even more in longitudinal studies, where psychopathology as well as sleep structure are carefully monitored, given the heterogeneity of diagnostic categories, frequent comorbidities in children and adolescents and the possible "progression" from anxiety disorders in early childhood to MDD and BPD during puberty.

Conclusions

The developing brain is extremely plastic. However, with this enhanced plasticity comes enhanced vulnerability (Anders, 1994). As mentioned above, there seems to be a specific time window during development where the brain is more prone to develop certain types of psychiatric illnesses. As a local increase of SWA not only

precedes the maturation of skills but also the structural maturation of cortical brain regions (Kurth et al., 2012), the detection of altered topography seems promising to recognize early perturbations during development which impinge on the final outcome. Understanding the pathophysiology of mental illnesses at an early stage paves the way towards new therapeutic approaches, which in return might benefit from the plasticity of the developing brain.

1.3. Structure of the Thesis and Aims

The overall aim of the thesis was to examine the relationship between sleep and cortical plasticity during development and in the context of natural environmental influences. Specific aims were designed to assess *1) particular aspects of sleep EEG characteristics for a better understanding of healthy and abnormal development*, as well as *2) effects of a moderate increase in altitude on sleep and learning related plasticity* - by setting the spotlight on the two main NREM sleep oscillations, slow waves and sleep spindles.

Part 1: Sleep During Development and in the Context of Early Onset Psychiatric Illnesses

Tesler, Gerstenberg, Huber (2013). **Developmental Changes in Sleep and their Relationships to Psychiatric Illnesses.** Published in *Current Opinion in Psychiatry*.

The first part of the thesis focuses on the most susceptible developmental stages - childhood and adolescence - with the aim to assess specific sleep EEG characteristics in both health and disease.

After a thorough description of the most widespread psychiatric illnesses in children and adolescents, the REVIEW article introduced previously, further discussed the interplay between sleep EEG characteristics and brain development in health and early-onset psychiatric illnesses. The following two original research articles focus on the two most disabling psychiatric illnesses emerging during a highly susceptible developmental period: Major Depressive Disorder and Schizophrenia, both disorders causing marked socioeconomic burden for the individual, their family and the entire community (Jenkins and Schumacher, 1999). Sleep disturbances have long been a major area of research in these disorders, however, no clear disease-specific patterns could be identified so far (Tesler et al., 2013). Recent evidence proposed that the sleep EEG presents a 'window through which adolescent brain development can be viewed' (Colrain and Baker, 2011a, Tarokh et al., 2011). This assumption triggered our next two studies, where we aimed to investigate specific sleep EEG characteristics (slow waves and sleep spindles) in children and adolescents diagnosed with Early Onset Schizophrenia and Major Depression. In the third study, we aimed to investigate longitudinally individual changes in SWA topography in

healthy children and adolescence and relate possible alterations to behavioural changes in a specific visuo-motor task.

1) **Tesler**, Gerstenberg, Franscini, Jenni, Walitza and Huber (2014). **Reduced Sleep Spindle Density in Early Onset Schizophrenia**. Submitted.

Schizophrenia is a severe mental disorder affecting approximately 1% of the worldwide population (NIMH, 2013). Its typical onset is during adolescence while early onset schizophrenia is a rather rare and more severe form of the disorder (Jacobsen and Rapoport, 1998, Thompson et al., 2001). Recently, thalamocortical deficits have been reported in adult schizophrenia patients (Ferrarelli et al., 2007, Ferrarelli et al., 2010, Wamsley et al., 2012). The sleep spindle, a thalamocortically generated phasic oscillation between 12-15 Hz during NREM sleep, reflects anatomical and functional differences of the thalamocortical system (De Gennaro and Ferrara, 2003, Lustenberger and Huber, 2012). Adult patients with schizophrenia show a remarkable decrease in sleep spindles (Ferrarelli et al., 2007, Ferrarelli et al., 2010, Wamsley et al., 2012). Furthermore, spindle deficits were associated with greater severity of positive symptoms (Ferrarelli et al., 2010). The spotlight of this study was on investigating by means of high density EEG, sleep spindles in adolescents diagnosed with schizophrenia and trying to relate any possible alterations in sleep spindles to symptom severity.

2) **Tesler**, Gerstenberg, Franscini, Jenni, Walitza and Huber (2014). **Increased Frontal Sleep Slow Wave Activity in Adolescents with Major Depression and their Unaffected Siblings**. Submitted.

The incidence of Major Depressive Disorder (MDD) rises substantially during adolescence (Green, 2005). Depression is associated with a high risk of suicide and is one of the leading causes of disease burden worldwide (Hyman et al., 2006, Nock et al., 2010). Sleep slow wave activity (SWA), the major characteristic of deep NREM sleep, was shown to be abnormal in depression (ex. (Frey et al., 2012) while sleep research revealed SWA to mirror both cortical restructuring and functioning (Born et al., 2006a, Tononi and Cirelli, 2006). The goal of this study was to assess characteristics of SWA topography in adolescents diagnosed with MDD. Additionally we included a group of unaffected siblings to further detect possible changes in individuals at high genetic risk with similar brain physiology.

3) Lustenberger, Mouthon, **Tesler**, Kurth, Ringli, Pugin, Huber. **Individual Slow Wave Activity Trajectories as a Marker for Brain Development**. In Preparation.

As SWA has been shown to best reflect both cortical restructuring and functioning of certain brain regions, in a next step, we investigated in a longitudinal study, changes of SWA during healthy adolescence, further relating these alterations to behavioural changes in a specific visuo-motor task. Since SWA decreases about 60% between 11 and 16 years (Campbell and Feinberg, 2009, Feinberg et al., 2006) it could be argued that during adolescent brain development intraindividual stability is not true. However, Tarokh and colleagues (Tarokh et al., 2011) recently demonstrated that trait-like aspects in the sleep EEG spectra exist across adolescence despite considerable cortical changes. We aimed to characterize the topographical aspects of SWA that remain stable during adolescence and aspects that change within and between subjects across this time period. Finally, there is good evidence, that SWA changes may be related or even involved in the maturation of specific skills (Kurth et al., 2012). We therefore further investigated the individual changes of SWA during adolescence in relation to behavioural changes in a specific visuomotor task.

Part 2: Sleep and Plasticity in the Context of Natural Environmental Influences

The next part of the thesis focuses on the effects of a moderate increase in altitude on sleep and learning in adults, in the context of external influences - moderate altitude.

4) Latshang, Lo Cascio, Stöwhas, Grimm, Stadelmann, **Tesler**, Achermann, Huber, Kohler and Bloch (2013). **Are Nocturnal Breathing, Sleep and Cognitive Performance Impaired at Moderate Altitude (1630-2590m)?** Published in *Sleep*.

Newcomers to high altitude (> 3000m) experience periodic breathing, sleep disturbances and impaired cognitive performance (Nussbaumer-Ochsner et al., 2012a). Whether similar adverse effects occur already at lower elevations is uncertain, although numerous lowlanders travel to more moderate altitudes for professional or recreational activities. In the next study, we evaluated the hypothesis that nocturnal breathing, sleep and cognitive performance of lowlanders are impaired already at moderate altitude.

5) **Tesler**, Latshang, Lo Cascio, Stadelmann, Stöwhas, Kohler, Achermann, Bloch, and Huber (2014). **Ascent to Moderate Altitude Impairs Overnight Memory Improvements.** Published in *Physiology and Behavior*.

Several studies showed that slow waves, seem crucial for the beneficial effects of sleep on memory performance (Abel et al., 2013, Rasch and Born, 2013). Therefore, in the next study, we investigated whether sleep dependent memory performance in healthy lowlanders can be dampened by natural environmental influences - moderate altitude. We chose to apply the visuo-motor rotation adaptation task, used in study 3, because previous studies reported showed clear sleep dependent memory benefits by using this specific task (Huber et al., 2004).

2

Research Part

2.1. Research Part 1:

Sleep During Development and in the Context of Early Onset Psychiatric Illnesses

2.1.1. Reduced Sleep Spindle Density in Early Onset Schizophrenia

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Submitted To: **Schizophrenia Research**

Abstract

Background

Schizophrenia is a complex neuropsychiatric disorder affecting 1% of the worldwide population. Its typical onset is during late adolescence. Abnormal brain connectivity, specifically thalamocortical alterations have been reported in adult schizophrenia. The sleep spindle, a thalamocortically generated phasic oscillation between 12-15 Hz during non-rapid eye movement (NREM) sleep, reflects the integrity of the thalamocortical system. Adult patients with schizophrenia show a deficit in sleep spindle density which seems to be associated with greater severity of positive symptoms. The focus of our study was on investigating sleep spindles in adolescents at an early stage of the disease by means of high-density electroencephalogram (hdEEG) and relating alterations to symptom severity.

Methods

All-night hdEEG was recorded in 9 patients (16.1±0.5y) and 9 controls (16.2±0.5y). Study inclusion for the patients required a diagnosis of schizophrenia, schizophreniform disorder or brief psychotic disorder according to DSM-IV. Actual symptom severity was assessed using the Positive and Negative Syndrome Scale (PANSS). Sleep stages were visually scored. Automatic spindle detection was performed, to assess spindle characteristics.

Results

Similar to adult patients, early onset schizophrenia patients showed significantly reduced sleep spindle density over a large area of centro-parietal and temporal brain regions. We found not only severity of positive symptoms to be negatively associated with spindle density but also general and total symptom scores.

Conclusions

Our findings indicate that sleep spindle deficits can already be detected in early onset schizophrenia, are associated with positive symptoms and may therefore be an electrophysiological marker for early detection of the disorder.

Introduction

Schizophrenia is a complex neuropsychiatric disorder affecting about 1% of the worldwide population (NIMH, 2013)(Cirelli and Tononi, 2008). Whereas schizophrenia in pre-pubertal children is rare, its incidence rises during adolescence (Thompson et al., 2001). Early onset schizophrenia spectrum disorders emerge between 12 and 18 years (EOS, including schizophrenia, schizoaffective, schizophreniform, delusional, brief psychotic and psychotic disorder not otherwise specified (NOS) and account for estimated 4-15% of all schizophrenias (Cannon et al., 1999, Hafner and Nowotny, 1995). EOS appears to be clinically and neurobiologically continuous with the adult onset of schizophrenia spectrum disorders (Jacobsen and Rapoport, 1998, Hollis, 2000, Schimmelmann et al., 2005) but may represent a more severe form of the disorder with more neurodevelopmental and cognitive deficits (Ballageer et al., 2005, Basso et al., 1997, Biswas et al., 2006, White et al., 2006). Characteristic positive symptoms such as delusions and hallucinations seem to be less intense and/or their expression less overt in children and adolescents, possibly contributing to the significantly longer lag between onset of disease and treatment in youth compared to adults (Hollis, 2003, Schimmelmann et al., 2007). Since EOS and a longer duration of untreated psychosis were shown to be associated with worse outcome (Schimmelmann et al., 2007, Clemmensen et al., 2012), research in children and adolescents is needed, with a major interest in uncovering underlying neuropathological mechanisms and detecting possible clinically useful markers to enhance early recognition and pave the way for specific treatment.

Based on recent imaging findings, schizophrenia has been described as a network disorder mediated by abnormal brain connectivity and disturbed neuronal communication (e.g. (Anticevic et al., 2013, Samartzis et al., 2014). Specifically, thalamocortical circuit alterations including deficits of sensory gating have been observed in adult schizophrenia (e.g. (Andreasen et al., 1994, Anticevic et al., 2013, Stephan et al., 2006, Ferrarelli and Tononi, 2011, Light and Braff, 1999, Tomitaka et al., 2000). The sleep spindle, a thalamocortically generated phasic oscillation between 12-15 Hz during non-rapid eye movement sleep (NREM), reflects anatomical and functional differences of the thalamocortical system (De Gennaro and Ferrara, 2003, Lustenberger and Huber, 2012). A considerable

decrease in sleep spindles was found in adult patients with schizophrenia in an early or chronic phase of disease compared to different control groups such as non-schizophrenic patients receiving antipsychotic medication, patients with a history of depression, and healthy controls (Ferrarelli et al., 2007, Ferrarelli et al., 2010, Wamsley et al., 2012, Manoach et al., 2014). First studies using high-densitiy EEG (hdEEG), a method that combines the high temporal resolution of EEG with high spatial resolution through an easy application method, further showed that the global reduction of sleep spindles over the entire cortex. However, most evident alterations were restricted to centro-parietal and temporal brain regions (Ferrarelli et al., 2007, Ferrarelli et al., 2010). Additionally, spindle deficits were associated with greater severity of positive symptoms (Ferrarelli et al., 2010). Therefore, sleep spindles are thought to be a reliable electrophysiological marker reflecting deficits in the integrity of the thalamocortical system in schizophrenia (Ferrarelli et al., 2007, Ferrarelli et al., 2010, Wamsley et al., 2012, Guller et al., 2012).

However, to our knowledge, so far spindles have not been investigated in EOS. Even though the typical onset of schizophrenia is in late adolescence, patients with this disorder are still quite rare under age 18 and present unique opportunities to study disease development (Thompson et al., 2001) while there is an even higher need in identifying and treating first episode patients early on. The focus of our study was to investigate sleep spindles in individuals with EOS by means of hdEEG and to relate any potential alterations in sleep spindles to symptom severity. According to previous findings in adult patients with chronic schizophrenia we expected (1) deficits in spindle density and (2) negative associations with severity of positive symptoms in our recently affected adolescents.

Methods and materials

Participants and clinical assessments

Nine children and adolescents (range: 13.6 - 17.6 years) meeting the criteria for an early onset schizophrenia spectrum disorder according to DSM-IV (American Psychiatric Association, 1994) (Steriade et al., 1993d)were recruited from in- and outpatient settings at the Department of Child and Adolescent Psychiatry, University of Zurich, Switzerland. DSM-IV Axis I diagnoses were confirmed by two

child and adolescent psychiatrists using the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID), a semi-structured interview for children and adolescents (Sheehan et al., 2010). Actual symptom severity of schizophrenia was further assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and total sum score as well as a positive symptoms score and a negative symptoms score were derived. Clinical and functional impairment were assessed using the Clinical Global Impression Scale (CGI) (Guy, 1976) and the Global Assessment of Functioning (GAF) (Hall, 1995). Pubertal status was assessed using the Tanner scale (Carskadon and Acebo, 1993). Depending on age the Wechsler Intelligence Scale for Children WISC IV (Daseking et al., 2007) (< age 17) or the Wechsler Adult Intelligence Scale Revised (Wechsler, 1981) (≥ age 17) was used to assess overall cognitive performance. Exclusion criteria were premorbid IQ <70, substance abuse/dependence within the last 6 months, and diagnosed neurological or sleep disorders.

Healthy controls were selected from ongoing studies in our lab. Controls were sex- and age-matched to the clinical group (range: 13.7 - 18.1). They underwent a telephone and questionnaire screening to exclude personal and family history of psychiatric disorders, chronic diseases, learning disabilities, sleep disorders and use of any medication.

Written informed consent was obtained from participants aged >18 or from the legal guardian of minors, and an additional written assent was obtained from each minor after careful explanation of the study methods and aims. The procedures were approved by the local ethics committee and the study was performed according to the Declaration of Helsinki. One week prior to the study, all participants were instructed to maintain regular sleep-wake schedules according to their habitual bedtimes. Their usual wake-sleep rhythm was monitored with self-reported sleep logs and wrist motor actigraphy (Actiwatch Plus, AW4, Cambridge Neurotechnology, Cambridge, England). 24 h before and during the course of the study, they were asked to refrain from alcohol and caffeine and to avoid naps.

Recording and preprocessing of EEG data

All EEG data were collected in the sleep laboratory of the University Children's Hospital Zurich with a hd-EEG system (Electrical Geodesic Sensor Net for a long-term monitoring, 128 channels). The nets were adjusted to the vertex and the cap electrodes were filled with gel electrolyte. Impedances were measured at the beginning of the recording and kept below 50kΩ. The sleep episode of each individual was scheduled according to habitual bedtimes.

EEG recordings were sampled at 500 Hz (filtered between .01-200 Hz) and referenced to the vertex (Cz). The data was then band-pass filtered between 0.5 and 50 Hz and downsampled to 128 Hz. The sleep stages were scored for 20-s epochs according to standard criteria (Iber, 2007). One person was involved in scoring the sleep stages and all scored nights were then reviewed by a second person to assure concordance of the scoring within and between individuals.

Artefacts were rejected on a 20 sec basis after visual inspection and if power exceeded a threshold based on a mean power value in the 0.75-4.5 and 20-30 Hz bands (Huber et al., 2000). The data was re-referenced to the average reference of all good quality EEG channels above the ears (109; of these, on average, 2 channels per individual were of insufficient quality).

Spindle detection

Sleep spindle detection was performed according to the detection algorithm of (Ferrarelli et al., 2007). We thereby focussed on the first hour of artefact-free NREM sleep. We selected this time interval because it includes the same number of epochs for all participants and belongs to the most consolidated part of sleep. The EEG signal was band-pass filtered between 12-15 Hz. A sleep spindle was detected in the rectified signal if the signal amplitude exceeded an upper threshold that was defined relative to the mean signal amplitude. An upper threshold of 5 times the mean signal was determined to result in the best spindle detection after visual inspection of spindle density values that were comparable with previous studies (e.g. (Dijk et al., 1993, Lustenberger, 2014, Nicolas et al., 2001). Beginning and end of sleep spindles were set when the signal around the peak amplitude dropped below a lower threshold (2 times the mean signal). We focussed our analysis on sleep spindle density (number per min NREM sleep), because this

measure is one of the most affected in adult patients with schizophrenia (e.g. (Ferrarelli et al., 2010, Wamsley et al., 2012).

Statistics

For topographical analysis we applied statistical nonparametric mapping (SnPM) using a suprathreshold cluster analysis to control for multiple comparisons and to define specific regions of interest (Ferrarelli et al., 2010, Nichols and Holmes, 2002). Thus, the neighbouring electrodes that were above/below a significant t-value of 2.12/-2.12 (corresponding to a p-value of 0.05 for n=9, degrees of freedom: 16) and exceeded the 95th percentile cluster size given by the permutation analysis were considered significant. Because of a small sample size, Spearman correlations were used to assess relationships between sleep spindle measures and psychotic symptoms. We also performed partial correlations to correct for the effects of IQ. All other variables were compared between the groups by using the Wilcoxon-Mann-Whitney test. Data variability is described as standard error of the mean (SEM). All analyses were performed with the software package MATLAB (MathWorks) and SPSS 16.0.

Results

Sample characteristics

The primary current diagnoses in the sample including adolescent patients were EOS schizophrenia (n=5; 55.6%), brief psychotic disorder (n=2; 22.2%) and schizophreniform disorder (n=1; 11.1%). Regarding comorbidities, , 3 (33.3%) had Attention-Deficit/Hyperactivity Disorder and 1 (11.1%) an autism spectrum disorder (Asperger Syndrome, average IQ). Five (55.6%) patients further met diagnostic criteria for an MDD in the past according to the MINI-KID. At the time of the sleep recordings, patients (n=9, mean age: 16.1±0.5 years) were in a stable phase of their illness or in recent (partial) remission in case of brief psychotic disorders, thus had an illness severity within the range of 'mildly' (3) to 'extremely ill' (7) (CGI, mean 5.3±0.4) and impaired global functioning ranging from 'good functioning in all areas with no more than everyday problems or concerns' (81) to 'inability to function in almost all areas' (21) (current GAF, mean 35.3±6.2) (see Table 1). The adolescents with EOS were mostly treated previously in an inpatient setting (n=6, 66.6%), followed by outpatient settings (n=3, 33.3%) and received mainly atypical

antipsychotic medication (n=7, 77.8%), and 3 (33.3%) were taking biperiden (Table 1) (Table1). Additionally, 5 patients (55.6%) received benzodiazepines. One parent was affected by schizophrenia but no other first degree family members of our patients showed a positive history for a schizophrenic disorder (data not shown). There were no significant group (patients and healthy controls) differences concerning age, gender and Tanner values. However IQ values were significantly lower in the patients compared to the healthy control group (Table 1).

Sample characteristics	**Patients (n=9)**	**Controls (n=9)**
Age, years, mean ± SEM	16.1 ± 0.5	16.2 ± 0.5
Sex, female, n (%)	3 (33.3)	3 (33.3)
IQ, mean ± SEM [a]	93.6 ± 7.6*	122.3 ± 4.2
Tanner Pubertyscale, mean ± SEM [b]	9.8 ± 0.4	9.2 ± 0.6
Current DSM-IV Diagnoses, n (%)		
Schizophrenia and other Psychotic Disorders		
Early onset Schizophrenia	5 (55.6)	N/A
Brief Psychotic Disorder	2 (22.2)	N/A
Schizophreniform Disorder	1 (11.1)	N/A
Lifetime DSM-IV Diagnoses, n (%)	6 (66.7)	N/A
History of Major Depressive Disorder	5 (55.6)	N/A
Attention-Deficit/Hyperactivity Disorder	3 (33.3)	N/A
Autism Spectrum Disorder	1 (11.1)	N/A
Actual Severity of Psychotic Symptoms, PANSS, mean ± SEM		
Total score	71.4 ± 6.3	N/A
Positive symptoms score	15.9 ± 1.8	N/A
Negative symptoms score	18.0 ± 1.7	N/A
General symptoms score	37.6 ± 3.7	N/A
Current Illness Severity: Clinical Global Impressions-Severity Scale, mean ± SEM	5.3 ± 0.4	N/A
Current Functional Level: Global Assessment of Functioning-Scale, mean ± SEM	35.3 ± 6.2	N/A
Duration of Illness; weeks; mean ± SEM	54.6 ± 19.1	N/A
Treatment setting and medication at time of the sleep recordings		none
Inpatients/Outpatients, n (%)	6/3 (67/33)	none
Duration of inpatient treatment prior to sleep recordings; weeks; mean ± SEM	10.1 ± 2.0	none
Receiving psychotropic medication, n (%)	7 (77.8)	none
Atypical Antipsychotics; Aripiprazole/Quetiapine/Risperidone	7 (77.8); 6/2/1 (67/22/11)	none
Benzodiazepines; Lorazepam	5 (55.6)	none

Others; Biperiden	3 (33.3)	None
Duration of treatment with antipsychotics; weeks; mean ± SEM	14.9 ± 9.4	None

Table 1: N/A= not available, * represent significant group differences. Differences were compared by using the Wilcoxon Mann-Whitney test. a. IQ: 1 EOS, 3 controls had missing data; b. Tanner: 1 EOS had missing data.

Sleep architecture

First, we examined visually scored sleep variables to evaluate the sleep quality of the samples. Sleep quality was good in all groups, thus showing high sleep efficiency (>85%). All other sleep stage measures were also comparable between the groups with no statistical significant differences (Table 2).

Visually scored sleep variables	**Patients (n=9)**	**Controls (n=9)**
Sleep latency (min)	23.9 ± 4.1	17.0 ± 2.1
REM sleep latency (min)	140.4 ± 27.2	128.9 ± 19.5
Wake after sleep onset (min)	28.4 ± 7.9	26.8 ± 8.3
Sleep stage 1 (%)	6.1 ± 0.8	7.7 ± 2.0
Sleep stage 2 (%)	51.0 ± 2.7	55.4 ± 0.7
Sleep stage 3 (%)	23.6 ± 5.3	18.8 ± 1.6
REM sleep (%)	19.3 ± 3.1	18.1 ± 1.0
Total sleep time (min)	424.7 ± 44.9	405.0 ± 22.6
Total time in bed (min)	427.8 ± 37.2	444.6 ± 19.7
Sleep efficiency (%)	87.2 ± 4.7	90.9 ± 2.5

Table 2: Visually scored sleep variables: Sleep latency in minutes, rapid eye movement (REM) sleep latency in minutes (mean±SEM), wake after sleep onset in minutes, sleep stage 1 in percent (%), sleep stage 2 in %, sleep stage 3 in %, total sleep time in minutes, total time in bed in minutes and sleep efficiency in %. The values were derived from visual scoring and relative values are calculated for each individual separately and then averaged for the entire group. There were no significant differences between the groups. Differences were compared by using the Wilcoxon Mann-Whitney test.

Spindle density in early onset schizophrenia patients compared to healthy controls

When contrasting spindle density between the patients and the group of healthy controls we found significant differences in spindle density over clusters of electrodes in the fast spindle frequency range between 13.75 and 14.5 Hz (Fig. 1). Therefore we next focused our analysis on this specific frequency range. We explored the topographical distribution of spindle density between patients and

healthy controls. The patients showed significantly reduced spindle density over a large area of centro-parietal and temporal brain areas (cluster of 22 electrodes) (Fig. 2).

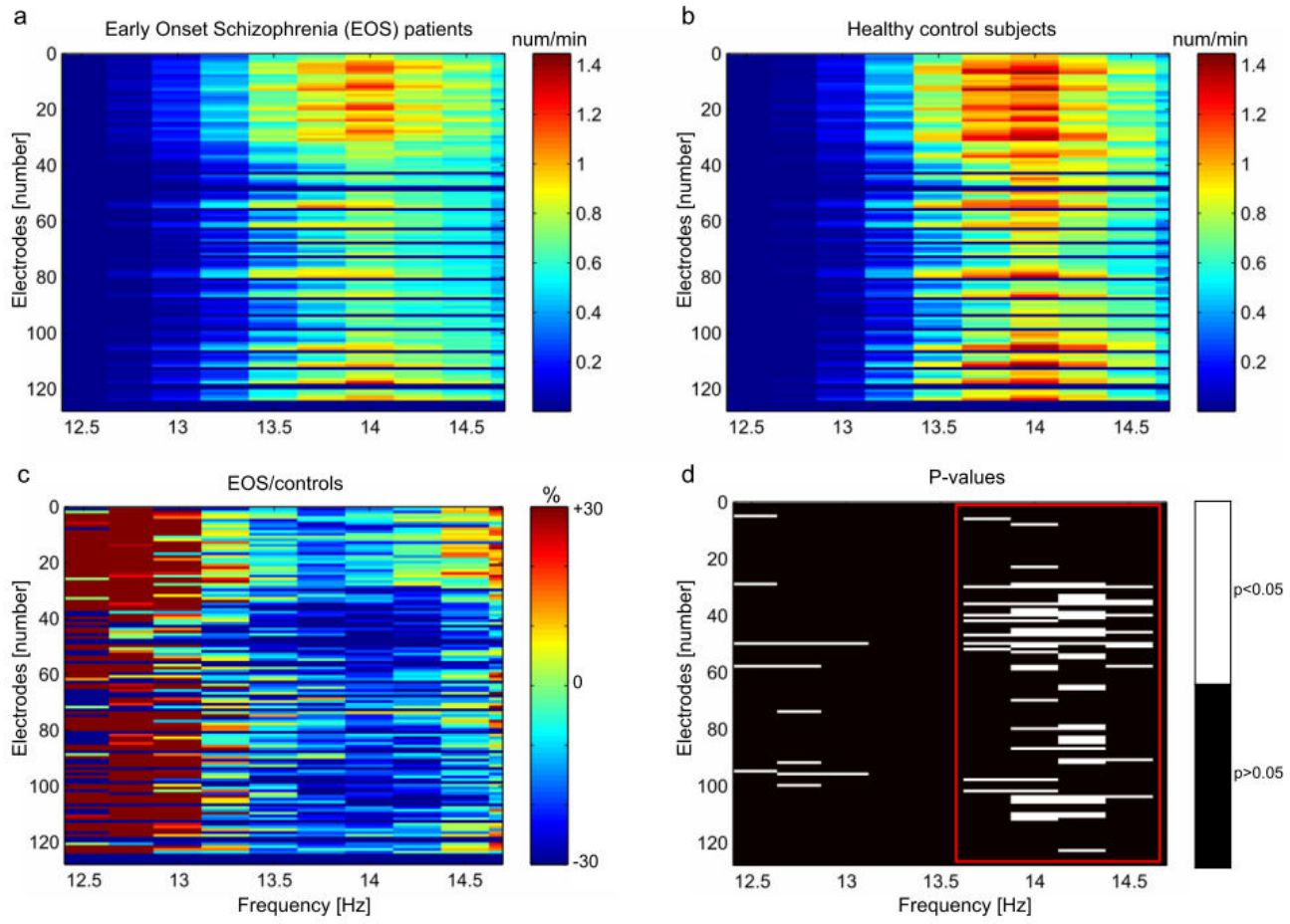

Figure 1: Heat plots of spindle density (num/min) of the first hour of NREM sleep for patients with EOS (a) (n=9) and age- and gender-matched healthy controls (b) (n=9). The ratio between patients with EOS and healthy controls is displayed in (c) and corresponding statistics in (d) for the differences between patients with EOS and healthy controls. The x-axis represents the frequency bins where sleep spindles were detected. The y-axis indicates the 128 EEG electrodes. The red rectangle highlights the frequency bins that showed significant (white bars, $p<0.05$) differences over pronounced clusters of electrodes.

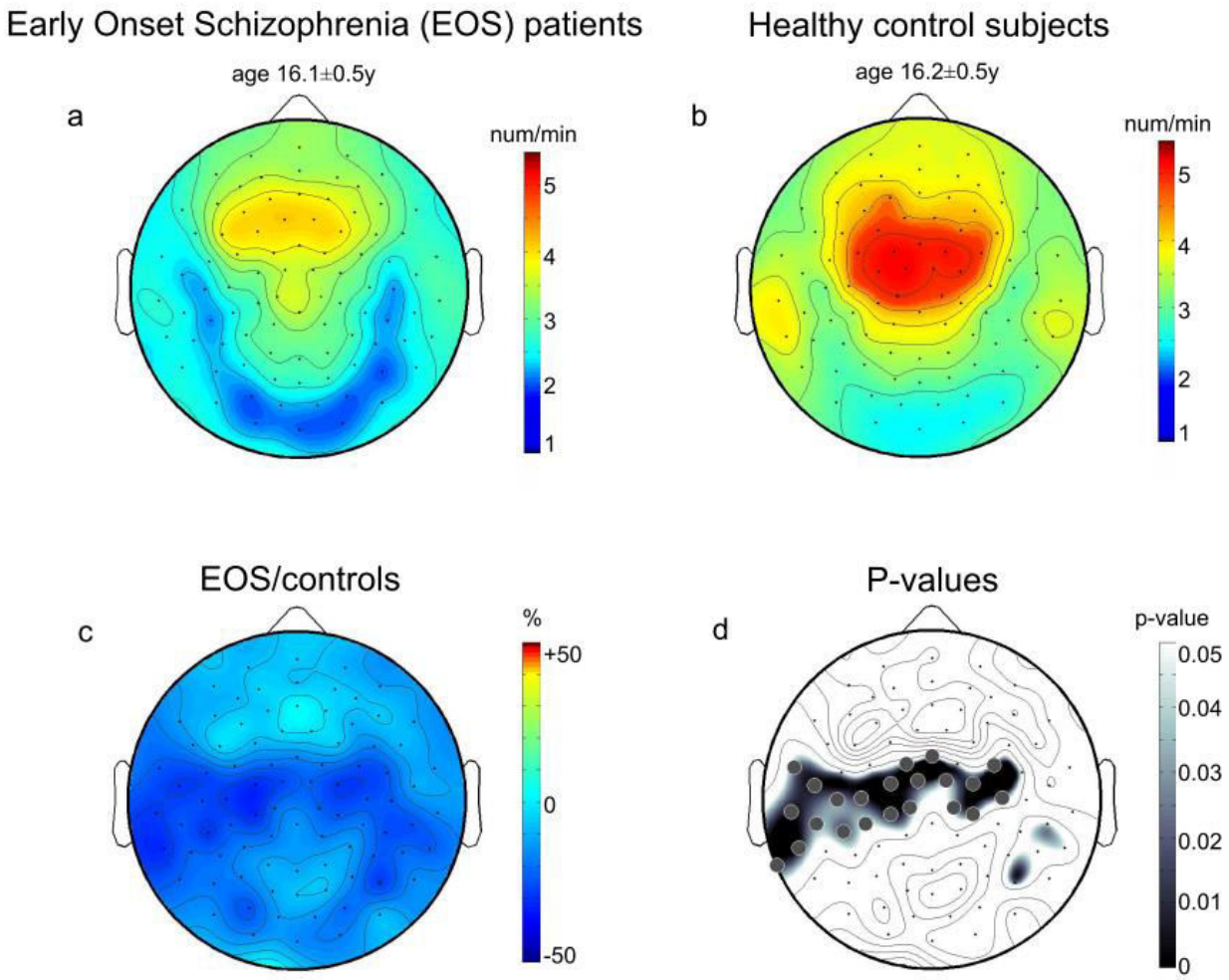

Figure 2: Topographical distribution of spindle density (num/min) for the first hour of NREM sleep plotted on the planar projection of the hemispheric scalp model for patients with EOS (a) (n=9) and age and gender-matched healthy controls (b) (n=9) for the frequency range between 13.75-14.5 Hz. Maxima are shown in red and minima in blue. Ratio between patients with EOS and healthy controls is shown in (c) and corresponding statistics in (d) for the differences between patients with EOS and healthy controls. Grey dots indicate electrodes showing significant differences after controlling for multiple comparisons.

Correlation with positive symptom severity

Based on these results, we investigated the relationship between spindle density and different psychotic symptoms. We found negative associations between positive symptoms and spindle density over a large area of the scalp (Fig. 3a). The results were similar when correlating the PANSS total and general symptom score with spindle density (data not shown). The topographical pattern of the correlations between positive symptoms and spindle showed similar global effects as when only contrasting spindle density between the two groups (Fig. 3a and 2c). When

correlating the mean of the significant electrodes with the positive symptoms we found a significant negative correlation (r=-0.75, p=0.02) (Fig. 3b, c). Figure 3d further shows individual patient ratings, illustrating that patients with the lowest spindle density values show highest positive symptoms in the PANSS positive symptoms score.

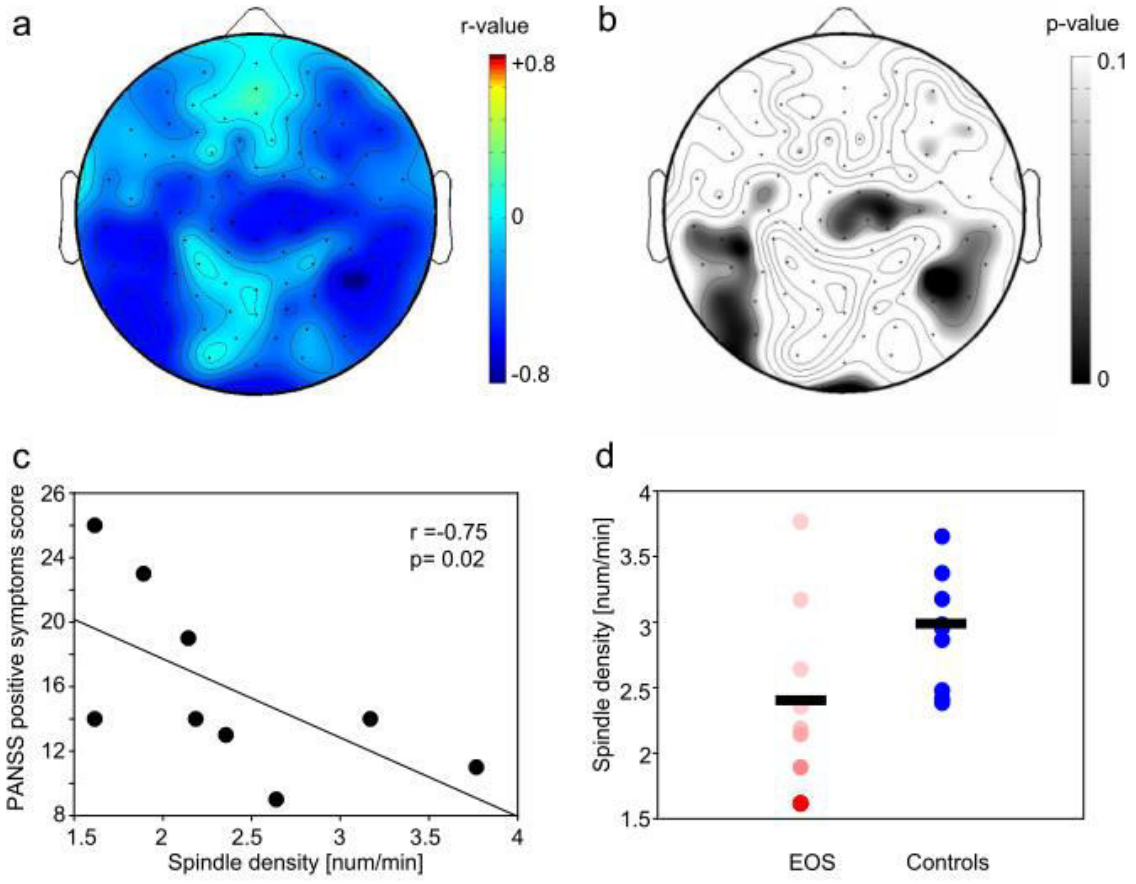

Figure 3: Illustration of the correlation between fast spindle density (13.75-14.5 Hz) and PANSS positive symptoms score for the schizophrenia patients. Topographical distribution of r-values and p-values for the first hour of NREM sleep are plotted on the planar projection of the hemispheric scalp model with negative correlations reflected in blue (n=9) (a, b). The correlation in (c) demonstrates the significant negative correlation between spindle density and PANSS positive symptoms score for the electrodes that showed significant values (r=-0.75, p=0.02). (d) further illustrates the individual spindle density values (num/min) of the electrodes that showed significant negative correlations with the PANSS positive symptoms score for patients with EOS in red and healthy controls in blue. The black lines represent the mean spindle densities over each group. For patients with EOS we further highlighted the individuals with the highest scores in the PANSS positive symptoms score in darker colours. Thus, patients with the highest PANSS positive symptoms scores showed lowest spindle densities.

Discussion

In this study we show for the first time that sleep spindle density was significantly reduced over a large area of centro-parietal and temporal brain regions in individuals affected by an early onset schizophrenia spectrum disorder. Furthermore, we found spindle deficits to be inversely related to positive symptom severity, suggesting that the spindle deficit could be a biomarker which is associated with presentation of positive symptoms and symptom severity also in adolescents.

Even though the patients were impaired by the disorder and received in the majority psychotropic medications, we did not find any alterations in sleep architecture between our young patients and healthy controls. The most widely reported sleep architecture abnormalities in adult schizophrenia patients - reduced slow wave sleep (Feinberg et al., 1969, Jus et al., 1968, Poulin et al., 2003) and abnormal rapid eye movement sleep (Poulin et al., 2003, Tandon et al., 1992, Yang and Winkelman, 2006) have not been consistently observed and have not withstood meta-analyses (Benca et al., 1992b, Chouinard et al., 2004). Up to now, relatively few studies have gone beyond sleep architecture (Wamsley et al., 2012). Results of the first studies investigating sleep characteristics, such as sleep spindles, in small samples of unmedicated adult patients showed variable results, with one study reporting increased spindle counts (Hiatt et al., 1985) or no alterations in spindle density (Poulin et al., 2003). These inconsistent results may reflect differences in methodology between the studies, e.g. in electrode placement, detection of spindles or stage of disease. However, subsequent studies using high density EEG (hdEEG) consistently reported reduced sleep spindle density in adult patients with recent onset of the disorder or a chronic schizophrenia (Ferrarelli et al., 2007, Ferrarelli et al., 2010, Wamsley et al., 2012, Manoach et al., 2014).

Our finding of reduced spindle density in a young population of mostly medicated patients with EOS is consistent with these findings. Furthermore, the negative relationship between positive symptoms and sleep spindle density is in line with previous findings in adult patients with schizophrenia (Ferrarelli et al., 2010).

Both density of sleep spindles and positive symptoms may depend on the anatomy and efficiency of the thalamocortical system, e.g. number, strength and myelinisation of thalamocortical fibers (Fogel et al., 2007, Miller, 1994). The reticular nucleus of the thalamus as the generator of sleep spindles (Kandel and Buzsaki, 1997, Steriade, 2006) plays a significant role in sensory input gating as well as processing and filtering of information (McAlonan et al., 2002). Patients affected by schizophrenia are thought to be overwhelmed with information and internal as well as external stimuli due to deficient thalamocortical circuits that may lead to the development of the clinical picture of delusions and hallucinations (Andreasen et al., 1994, Crail-Melendez et al., 2013). Thus, anatomical and functional variations of the thalamus may be associated with differences in sensory gating and filtering of information that may unclose the emergence of positive symptoms and be paralleled by a reduction in sleep spindles. Therefore, spindle density seems to be a promising marker to aid the diagnostic process for schizophrenia spectrum disorders, not only in adults, but already in adolescents.
Two recent studies investigating density of sleep spindles support this notion. One study demonstrated that first degree relatives of patients with schizophrenia showed a trend for reduced spindle density compared to healthy controls; however to a lesser extent than the affected sample (Manoach et al., 2014). Another study revealed that higher scores on a magical ideation scale reflecting a proneness to delusion-like beliefs, were associated with a reduction in sleep spindle density. Hereby a continuum model was proposed, suggesting that unusual but non-clinical beliefs may represent a milder form of the clinical positive symptoms found in severe mental illness (Lustenberger, 2014, van Os et al., 2009).

The difference in spindle density between the patients and healthy controls was restricted to the high spindle frequency range (13.75-14.5 Hz). The topographical distribution of spindle density revealed that compared to healthy controls our patients showed less sleep spindles almost over the whole cortex. Nonetheless, only electrodes over centro-parietal and temporal brain areas reached significance level. When focussing on the first hour of NREM sleep, also Ferrarelli et al. (2007) (Ferrarelli et al., 2007) described a spindle reduction in adult patients with schizophrenia that was restricted to the high frequency range (13.75-15 Hz) and to a central cluster with maxima over prefrontal and centroparietal regions. Cortical

topography of sleep spindles demonstrate that the majority of sleep spindles over centro-parietal and temporal brain regions are around 14 Hz whereas slow spindles around 12 Hz are most pronounced over frontal regions (De Gennaro and Ferrara, 2003). Therefore, in schizophrenia, thalamocortical circuits with projections to or receiving projections from centro-parietal and temporal regions may be specifically impaired and of major interest for further investigations. This region-specific aspect is further underlined by our finding, that the inverse correlation of symptom severity and spindle density was visible over the whole cortex but only specific electrodes over centro-parietal and temporal brain areas reached significant level. Interestingly, in contrast to adult patients with chronic schizophrenia (Ferrarelli et al., 2010) we also found the general symptoms score to be inversely correlated to the spindle density in our adolescent sample. With a total PANSS score of 71, our sample may be classified between the two adult samples (scores 55 (Wamsley et al., 2012)and 89 (Ferrarelli et al., 2010)) but in absence of subscale scores in the previous studies (Ferrarelli et al., 2010, Wamsley et al., 2012), we can only hypothesize, that our finding may in part be due to the diverse clinical picture of EOS compared to schizophrenia with adult onset. Working with the PANSS that was mainly developed for adults, a factoranalytic approach in children who were reassessed 42 years later revealed that schizophrenic symptoms at onset did not group into separate positive/negative but different dimensions such as a cognitive component (including 5 general symptom items) and factors reflecting different social behaviours (Bunk et al., 1999). Due to our small sample size, we did not correlate single items with spindle density, but Ferrarelli et al. (2010) could show that beside hallucinations, also a formal thought disorder, conceptual disorganisation, was inversely associated with spindle density in adult patients (Ferrarelli et al., 2010). Since several studies have underlined the importance of sleep spindles in neurocognitive domains and memory processing (Rasch and Born, 2013, Geiger et al., 2011), future studies assessing reported cognitive disturbances, neurocognitive deficits as well as sleep spindle measures may be helpful to yield further insight into this complex interaction and possible age- and maturation specific aspects.

In comparison to our healthy control sample with above-average IQ, our EOS sample showed significantly reduced IQ. Nonetheless we could not find any

associations between IQ values and spindle density in our sample, finding that is in line with previous findings in an adult sample (Ferrarelli et al., 2010). These results support the notion that spindle deficits in schizophrenia are unrelated to reduced general cognitive ability. Still, possible correlations between spindle reduction and deficits in specific neurocognitive aspects such as attention and memory-related functions in schizophrenia are not excluded (Gur et al., 2007). Variations of sleep spindles have been shown to be associated with cognitive dysfunctions (Fogel et al., 2007) and in adult patients with schizophrenia worse scores of attention, psychomotor speed tasks and estimated verbal IQ were associated with lower spindle density (Manoach et al., 2014, Wamsley et al., 2012).

Discussing these present results we should keep in mind some limiting factors of our study. Beside schizophrenia, in this sample also schizophrenia-spectrum disorders were included that may be diagnostically less stable and different neurobiological pathways may contribute to their emergence. The number of individuals is rather low and we included one patient with a chronic medical condition that may have affected brain development. Still, IQ was above 70, no neurological disorder was diagnosed and excluding this patient from our analyses did not change our findings. Furthermore, since it was a naturalistic study, the use of psychotropic medication was present in most of our patients, and thus we do not know how these substances might have influenced our results. However, we did not find any association between the duration of treatment with antipsychotics and spindle density (data not shown). Moreover, Ferrarelli et al. (2010) (Ferrarelli et al., 2010) found sleep spindles to be reduced in adult patients with schizophrenia compared to non-schizophrenic patients taking antipsychotic medication and a recent study in healthy individuals without any medication found a relationship between spindle density and magical ideation. Additionally, the similar sleep architecture between our young patient group and the healthy controls points towards an effect irrespective of medication. These findings support that the reduced spindle densitiy is rather associated to the psychopathology of schizophrenia than caused by medication.

Taken together, our results indicate that spindle deficits over centro-parietal and temporal regions may be a potential biological marker for schizophrenia spectrum

disorders irrespective of age at onset. An early recognition of schizophrenia spectrum disorders during adolescence is essential, since the changes that take place during this sensitive developmental period can have a huge impact on later adult life (Cyranowski et al., 2000, Patton and Viner, 2007).

The longitudinal and combined detection of alterations in symptomatology and spindle density may contribute to the understanding of anatomical and functional alterations of the thalamocortical system. Future studies may further investigate the relationship between sleep spindle measures, topographical distribution, and different dimensions of psychopathology such as the possible continuum of subtle cognitive disturbances to formal thought disorder and neurocognitive deficits or of magical ideation to delusions to provide insight into the neural circuits underlying such alterations.

2.1.2. Increased Frontal Sleep Slow Wave Activity in Adolescents with Major Depression and their Unaffected Siblings

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Abstract

Importance

The incidence of Major Depressive Disorder (MDD) substantially rises during the vulnerable developmental phase of adolescence, where major cortical restructuring is taking place. How healthy development and aberrant cortical functioning in depressed adolescents are mutually related is a key issue. Sleep slow wave activity (SWA, EEG power in the 1-4.5 Hz range), the major electrophysiological characteristic of deep sleep, might represent a promising endophenotype because it mirrors both cortical restructuring and functioning.

Objective

The goal of this study was to assess specific characteristics of SWA topography in adolescents diagnosed with MDD (n=15) with high densitiy EEG. Additionally, we included a group of unaffected siblings (n=7) to detect possible changes in individuals at high familial risk.

Design, setting, and participants

Fifteen patients (15.1±0.3 years, age range: 12.9-16.6 years) meeting the criteria of MDD according to DSM-IV/5 were recruited from the Department of Child and Adolescent Psychiatry. DSM Axis I diagnoses were based on the Mini International Neuropsychiatric Interview for Children and Adolescents. Seven unaffected siblings (14.9±1.2 years, age range: 10.2-18.3 years) were further also studied. Twenty-two healthy controls were selected from ongoing studies. Controls were sex- and age-matched to both, the patients with MDD as well as their siblings. All night high-density EEG was recorded in all participants at the University Children's Hospital Zurich.

Main outcomes and measures

High-density EEG (128 electrodes) was performed. Sleep stages were visually scored and power maps were calculated based on the average SWA of the first and last hour of non-rapid eye movement (NREM) sleep.

Results

Sleep architecture did not differ between the groups. Depressed adolescents exhibited more SWA (35.2±1.4% ($p<0.05$)) in a cluster of eight frontal electrodes compared to healthy controls. A similar increase of frontal SWA was found in unaffected siblings. This frontal increase in SWA was restricted to the low frequency range (<2 Hz) and remained stable across the night for both groups.

Conclusions and relevance

Higher frontal SWA in adolescents with depression and their unaffected siblings might reflect a neurobiological correlate of increased familial risk for early onset depression.

Introduction

Depression is one of the leading causes of disease burden worldwide (Hyman et al., 2006). It is a highly disabling, often chronic illness, associated with increased risk of suicide (Ferrari et al., 2013). The incidence of Major Depressive Disorder (MDD) is relatively low in younger children (Kessler et al., 2001), yet rises substantially throughout adolescence (Green, 2005), with an almost twofold increase of the lifetime prevalence between age 13 (8.4%) and 18 (15.4%) (Merikangas et al., 2010). This increasing emergence of depression during a vulnerable developmental period (Blakemore and Choudhury, 2006) coincidences with pronounced structural and functional modifications in the brain (Paus et al., 2008). These alterations are not paused during sleep, in contrast sleep is considered an active process, also in the development of the central nervous system (Hobson and Pace-Schott, 2002b) and increasing evidence suggests a close relationship between sleep and cortical plasticity (Diekelmann and Born, 2010, Sejnowski and Destexhe, 2000, Steriade and Timofeev, 2003, Tononi and Cirelli, 2014). Specifically, the slow fluctuations of cortical activity during deep sleep, visible in the surface electroencephalography (EEG) as slow waves (Steriade et al., 1993c) and measured as slow wave activity (SWA; frequency range 0.75 - 4.5 Hz), have been shown to mirror the extensive synaptic reorganization of cortical areas from early childhood to late adolescence. SWA is further closely related to efficient cognitive functioning (Born et al., 2006b, Sejnowski and Destexhe, 2000, Steriade and Timofeev, 2003, Tononi and Cirelli, 2014) and may be involved in the consolidation of memories related to emotions, thoughts and actions (Rasch and Born, 2013). Reported sleep disturbances as a core symptom of MDD and altered sleep structure have long been a major area of research in depression. In depressed adults, alterations in sleep structure such as decreased slow wave sleep are quite consistently observed (Benca et al., 1992a, Borbely and Wirz-Justice, 1982) and were proposed as biomarkers predicting the response to treatment with a specific antidepressant or even the course of the disorder for several years (Steiger and Kimura, 2010). Findings related to sleep structure in youth have been discussed controversial so far (Gregory et al., 2009, Riemann and Voderholzer, 2003, Tesler et al., 2013).

As a major marker of the homeostatic regulation of sleep, several studies investigated sleep SWA in the context of MDD. Results of these studies in adults are

inconsistent showing reduced (Armitage et al., 2001, Armitage et al., 2000), but also increased SWA at baseline (Frey et al., 2012, Schwartz et al., 2001) as well as after sleep deprivation (Frey et al., 2012, Sejnowski and Destexhe, 2000). To our knowledge, only one study up to now focused on SWA in depressed adolescents, reporting lower SWA in depressed males compared to controls (Lopez et al., 2012). However, none of the previous studies used high-density EEG (hdEEG) to investigate topographical aspects of the SWA distribution.

Since several studies suggested that the genetic influence in adolescent-onset depression may be even higher compared to adult-onset (Thapar et al., 2012, Todd et al., 1993), the inclusion of unaffected siblings with similar basic brain physiology and analogous environment are needed to further discern underlying mechanisms of the disease and enhance the identification of depression endophenotypes (Savitz and Drevets, 2009, Hasler and Northoff, 2011).

Consequently, the main emphasis of our study was to investigating SWA topography in adolescents diagnosed with MDD and their unaffected siblings by means of hdEEG, which enables to detect subtle abnormalities in both cortical restructuring and functioning.

Methods and materials

Participants and clinical assessments

Fifteen children and adolescents (age range: 12.9 - 16.6 years) meeting criteria of a Major Depressive Disorder, single episode or recurrent, according to DSM-IV and DSM-5 (Association, 1994, Association, 2013) were recruited from in- and outpatient settings at the Department of Child and Adolescent Psychiatry, University of Zurich, Switzerland. Seven unaffected siblings of the patients (age range: 10.2 - 18.3 years) were further included in the study.

For the depressed patients and their siblings, DSM Axis I diagnoses were assessed by two child and adolescent psychiatrists using the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID), a semi-structured interview for children and adolescents (Sheehan et al., 2010). For the depressed group past psychiatric illness and treatment information was also

obtained from the parent/guardian and augmented by medical chart information. The actual symptom severity was further assessed using the Children's Depression Rating Scale-Revised (CDRS-R) (Poznanski, 1996) (Keller et al., 2011). The total sum score as well as five subscores (Guo et al., 2006) (observed depressive mood; anhedonia; morbid thoughts; somatic symptoms; reported depressive mood) were derived. "MDD in remission" was defined as CDRS-R sum score lower than 28 (March et al., 2007) at time of the sleep recordings, but fulfilling criteria of MDD within the past 2 months. "MDD in the past" was defined as a previous episode of recurrent depressive disorder or in siblings, as meeting criteria for a MDD in the past (at least one year before the sleep recordings). Clinical and functional impairment were assessed using the Clinical Global Impression Scale (CGI) (Guy, 1976) and the Global Assessment of Functioning (GAF) (Hall, 1995). Pubertal status and parental socio-economic status of all participants were assessed using the Tanner scales (Carskadon and Acebo, 1993) and the Hollingshead socio economic state scale (Hollingshead, 1975), respectively. The Wechsler Intelligence Scale for Children WISC IV (Daseking et al., 2007) and for 2 control participants in addition the TONI-IV (Brown, 1997), were used to assess overall cognitive performance.

Exclusionary psychiatric disorders for the depressed group were: schizophrenia, bipolar disorder, autism spectrum disorder, eating disorder, and substance-dependence. Other comorbidities frequently detected in adolescents with MDD such as anxiety or disruptive behaviour disorders were permitted (Kessler et al., 2003), provided that MDD was the primary diagnosis. Exclusion criteria for all participants were: an intelligence quotient (IQ) < 80 (Brown, 1997, Daseking et al., 2007) and a medical/neurological condition known to affect the brain.

Twenty-two healthy controls were selected from ongoing studies in our lab. Controls were sex- and age-matched to both our depressed group (age range: 12.8 - 16.4 years) as well as to their unaffected siblings (age range: 10.2 - 18.9 years). They underwent a telephone and questionnaire screening to exclude personal and family history of psychiatric disorders, chronic diseases, learning disabilities, sleep disorders and use of psychotropic medication.

Written informed consent was obtained from participants aged >18 or from the legal guardian of minors, and an additional written assent was obtained from each minor after careful explanation of the study methods and aims. The procedures were approved by the local ethics committee and the study was performed according to the Declaration of Helsinki. One week prior to the study, all participants were instructed to maintain regular sleep-wake schedules according to their habitual bedtimes. Their usual wake-sleep rhythm as well as the reported sleep latency were monitored with self-reported sleep logs and objective wake-sleep rhythm was additionally assessed by wrist motor actigraphy (Actiwatch Plus, AW4, Cambridge Neurotechnology, Cambridge, England). Twenty-four hours before and during the course of the study, they were asked to refrain from alcohol and caffeine and to avoid naps.

Recording and preprocessing of EEG data

All EEG data were collected in the sleep laboratory of the University Children's Hospital Zurich with a high-density-EEG device (Electrical Geodesic Sensor Net for a long-term monitoring, 128 channels). The nets were adjusted to the vertex and the cap electrodes were filled with gel electrolyte. Impedances were measured at the beginning of the recording and kept below 50kΩ. The sleep episode of each participant was scheduled according to habitual bedtimes. Thus participants came to the sleep laboratory 2 hours before their habitual bedtime, in order to allow enough time to prepare them for the night recordings.

EEG recordings were sampled at 500 Hz (filtered between 0.01-200 Hz) and referenced to the vertex (Cz). The data was then band-pass filtered between 0.5 and 50 Hz and downsampled to 128 Hz. After visually scoring for sleep stages (20 sec epochs, American Academy of Sleep Medicine standard criteria (Iber, 2007), NREM sleep episodes were defined according to standard criteria (Feinberg and Floyd, 1979, Rechtschaffen, 1968). One person was involved in scoring the sleep stages and all scored nights were then reviewed by a second person to assure concordance of the scoring within and between participants. Artefacts were rejected on a 20 sec basis after visual inspection and if power exceeded a threshold based on a mean power value in the 0.75-4.5 and 20-30 Hz bands (Huber et al., 2000).

After exclusion of EEG channels of insufficient quality (on average, 2 channels per participant) the data was rereferenced to average reference.

EEG power analysis, spectral analysis and statistics

We performed spectral analysis of consecutive 20 sec epochs (fast Fourier transform routine, Hanning window, averages of five 4 sec epochs, frequency resolution of 0.25 Hz). To assess topographical differences between the groups, SWA was calculated as mean power in the range of 1 - 4.5 Hz during the first 60 min of NREM sleep stage 2 and 3. We selected this time interval to account for differences in sleep episode durations and because it belongs to the most consolidated part of sleep. To investigate overnight changes in SWA, we further compared alterations of SWA from the first to the last hour of NREM sleep. To assess significant topographical differences in SWA between the groups we applied statistical nonparametric mapping (SnPM) using a suprathreshold cluster analysis for multiple comparisons (Nichols and Holmes, 2002). Anatomical localization of electrodes was verified in previous studies (Kurth et al., 2010b, Ringli et al., 2013) using magnetic resonance imaging (MRI) and the positioning software SofTaxic Optic (EMS Inc). Electrodes were digitized and co-registered with the subject's MRI (for details see (Kurth et al., 2010b)). We found a high agreement between the alignment of the electrode location and the corresponding anatomical area in a previous study (Kurth et al., 2012). Two-way analysis of variance (ANOVA) was performed with group (depressed - healthy controls vs. unaffected siblings - healthy controls) and gender (male vs. female) as independent factors and SWA as dependent factor. Mixed model ANOVA was used to compare the alterations in SWA from the first to the last hour of NREM sleep (SWA as a dependent repeated within-subject factor, group as an independent between-subject factor). Pearson product-moment correlation coefficients were calculated to assess relationships between SWA and actual symptom severity (CDRS-R total score and subscores) in the affected sample. All other variables were compared between the groups by using unpaired t-tests. Data variability is described as standard error of the mean (SEM). All analyses were performed with the software package MATLAB (MathWorks) and SPSS 16.0.

Results

Sample characteristics

At the time of the sleep recordings, patients with adolescent depression (n=15, mean age: 15.1±0.3 years) had a moderate to severe illness severity (CGI, mean 4.5±0.2) and impaired global functioning (current GAF, mean 52.8±4.0) (Table 1). Three of the depressed individuals (20%) met criteria within the past 2 months according to DSM-IV and DSM-5, but showed CDRS-R sum scores lower than 28The actual severity of depressive symptoms ranged between 21 and 61 (mean score 41.7±3.0). Most patients (n=9, 60%) had one or more current comorbid disorders, mainly anxiety disorders (n=6, 40%) such as social phobia (n=4, 27%), specific phobia (n=2, 13%) and one patient additionally met criteria for panic disorder. Four patients (27%) had comorbid attention deficit/hyperactivity disorder, and two of them also met criteria for a conduct disorder. The depressed patients were mostly treated in an inpatient setting (n=8, 53%), followed by day-clinics (n=4, 27%) and outpatient settings (n=3, 20%). The majority of the patients received Selective-Serotonin-Reuptake-Inhibitors (SSRIs) (n=9, 60%) (Table 1). One patient additionally received a tricyclic antidepressant (Mirtazapine), one patient was treated with atypical antipsychotic medication (Quetiapine) and five patients (n=5, 33%) were medication naive (Table 1). The unaffected siblings (n=7, mean age: 14.9±1.2 years) did not meet criteria for a current or remitted MDD, whereas 2 of them (29%) met criteria for MDD in the past without receiving any kind of therapeutic intervention. Two siblings (29%) met criteria for a specific phobia at the time of the sleep recordings (Table 1). Regarding family history of psychiatric illnesses, one mother and one father of two different patients were diagnosed with MDD in the past (data not shown). There were no significant group differences concerning age, gender, IQ, SES and Tanner values.

Sample Characteristics	**Depressed (n=15)**	**Controls Depressed (n=15)**	**Unaffected Siblings (n=7)**	**Controls Unaffected Siblings (n=7)**
Age, years, mean ± SEM	15.1 ± 0.3	15.3 ± 0.3	14.9 ± 1.2	15.4 ± 1.3
Sex, female, n (%)	8 (53)	8 (53)	5 (71)	5 (71)
IQ, mean ± SEM	113.5 ± 2.6	115.8 ± 6.1[a]	109.9 ± 1.7[b]	112.8 ± 5.9[c]
Socio Economic State Scale, mean ± SEM	5.3 ± 0.4	3.5 ± 0.4[d]	4.7 ± 0.7	3.5 ± 0.4[e]
Tanner Pubertyscale, mean ± SEM	9.7 ± 0.3	9.5 ± 0.8[f]	8.0 ± 1.5	8.4 ± 1.7
Current DSM-IV Diagnoses, n (%)				

Major Depressive Disorder (MDD)	12 (80)	N/A	0	N/A
MDD in Remission	3 (20)	N/A	0	N/A
Anxiety Disorders	6 (40)			
Panic Disorder	1 (7)	N/A	0	N/A
Social Phobia	4 (27)	N/A	0	N/A
Specific Phobia	2 (13)	N/A	2 (29)	N/A
Disruptive Behaviour Disorders[g]	4 (27)			
ADHD	4 (27)	N/A	0	N/A
Conduct Disorder	2 (13)	N/A	0	N/A
Lifetime DSM-IV Diagnoses, n (%)				
MDD in the past	6 (40)	N/A	2 (29)	N/A
Panic Disorder	2 (13)	N/A	0	N/A
Actual Severity of Depression, CDRS-R Sum Score, mean ± SEM	41.7 ± 3.0	N/A	N/A	N/A
Illness Severity: Clinical Global Impressions-Severity Scale, mean ± SEM	4.5 ± 0.2	N/A	1.0 ± 0.0	N/A
Current Functional Level: Global Assessment of Functioning-Scale, mean ± SEM	52.8 ± 4.0	N/A	89.7 ± 2.9	N/A
Treatment setting and medication at time of the sleep recordings				
Inpatients/Day-Clinics/Outpatients (%)	8/4/3 (53/27/20)	none	None	None
Receiving psychotropic medication, n (%) [h]	10 (67)	none	None	None
Medication class, n (%)				
Selective-Serotonin-Reuptake-Inhibitors[i]	9 (60)	none	None	None
Noradrenergic and Specific Serotonergic Antidepressant (Mirtazapine)	1 (7)	none	None	None
Atypical Antipsychotic (Quetiapine)	1 (7)	none	None	None

Table 1: Sample characteristics: N/A= not available; MDD in remission = defined as Children's Depression Rating Scale Sum Score < 28 at time of the sleep recordings, but fulfilling criteria of MDD within the past 2 months, MDD in the past = defined as a previous episode in recurrent depressive disorder or in siblings, as meeting criteria for a MDD in the past (at least one year before the sleep recordings). Data available for a=8, b=3, c=5, d=2, e=2, f=9 adolescents.

g=The total number of patients in one diagnostic category can be smaller than the sum of the individual diagnoses due to comorbidity.

h=The total number of patients receiving psychotropic medication is smaller than the sum of the agents due to one patient who received Sertraline and Mirtazapine.

i=Selective-Serotonin-Reuptake-Inhibitors include Fluoxetin (n=4), Citalopram (n=1), Sertraline (n=4).

Differences were compared by using two tailed, unpaired Student's t-test.

Sleep architecture

First, we examined visually scored sleep variables to evaluate the sleep quality of the samples. Sleep quality was comparably good in all groups, thus showing high sleep efficiency (>85%). All other sleep stage measures, such as sleep latency, rapid-eye movement (REM) sleep latency, wake after sleep onset, sleep stage 1, 2 and 3, REM sleep, total sleep time, total time in bed as well as sleep efficiency were also comparable between the groups with no significant differences (Table 2). Only the self-reported sleep latency was significantly higher in depressed adolescents compared to controls (31.2 ± 6.9 vs. 19.0 ± 2.3 min, p=0.05) and showed the same trend in siblings compared to controls (22.9 ± 3.3 vs. 18.3 ± 3.1 min, p=0.07) (Table 2).

Visually scored sleep variables	**Depressed mean ± SEM**	**Controls Depressed mean ± SEM**	**Unaffected Siblings mean ± SEM**	**Controls Unaffected Siblings mean ± SEM**
Sleep latency (min)	18.5 ± 3.6	22.5 ± 4.5	37.3 ± 11.4	17.7 ± 2.6
REMS latency (min)	109.1 ± 8.3	134.7 ± 17.1	152.9 ± 29.5	66.6 ± 1.2
Wake after sleep onset (min)	13.2 ± 3.4	21.4 ± 4.8	36.8 ± 15.2	37.1 ± 9.0
Sleep stage 1 (%)	6.3 ± 1.0	7.1 ± 0.6	6.8 ± 0.8	7.6 ± 1.7
Sleep stage 2 (%)	50.3 ± 1.7	53.1 ± 0.9	45.2 ± 2.4	53.5 ± 2.4
Sleep stage 3 (%)	22.7 ± 2.0	22.9 ± 1.3	25.4 ± 2.3	19.0 ± 3.1
REMS (%)	20.7 ± 1.8	16.9 ± 1.2	22.6 ± 1.7	19.9 ± 0.3
Total sleep time (min)	429.6 ± 23.8	392.2 ± 35.1	403.4 ± 17.2	388.0 ± 17.9
Total time in bed (min)	462.4 ± 21.4	433.7 ± 36.6	473.1 ± 26.4	438.2 ± 27.0
Sleep efficiency (%)	92.0 ± 2.0	90.0 ± 1.5	86.1 ± 3.8	89.1 ± 2.0
Reported sleep latency (min)	31.2 ± 6.9**	19.0 ± 2.3	22.9 ± 3.3*	18.3 ± 3.1

Table 2: Visually scored sleep variables. Sleep latency in minutes, rapid eye movement (REM) sleep latency in minutes, wake after sleep onset in minutes, sleep stage 1 in percent (%), sleep stage 2 in %, sleep stage 3 in %, total sleep time in minutes, total time in bed in minutes and sleep efficiency in %. The values were derived from visual scoring and relative values are calculated for each subject separately and then averaged for the entire group. Differences were compared by using two tailed, unpaired Student's t-test. ** represent significant differences (p≤0.05) and * represents a trend (p=0.07).

Topographical distribution of SWA in depressed adolescents compared to healthy controls

To investigate the topography of sleep SWA in adolescents with depression we calculated EEG power maps for each group. The topographical distribution of absolute values of SWA in the first hour of NREM sleep showed regional differences with maxima over the frontal cortex and minima over the temporal lobes (Figure 1a and 1b). When contrasting the maps, we found higher SWA over the frontal cortex in depressed adolescents (Figure 1c and 1d). Compared to age- and gender-matched controls, depressed adolescents exhibited 35.2% (±1.4%, $p<0.05$) more SWA in a cluster of 8 frontal electrodes (SnPM, see methods for details; Figure 1d). This frontal predominance of SWA in depressed adolescents can also be found in single individuals (see Figure 1a for 3 individuals with different ages and their age matched control in Figure 1b).

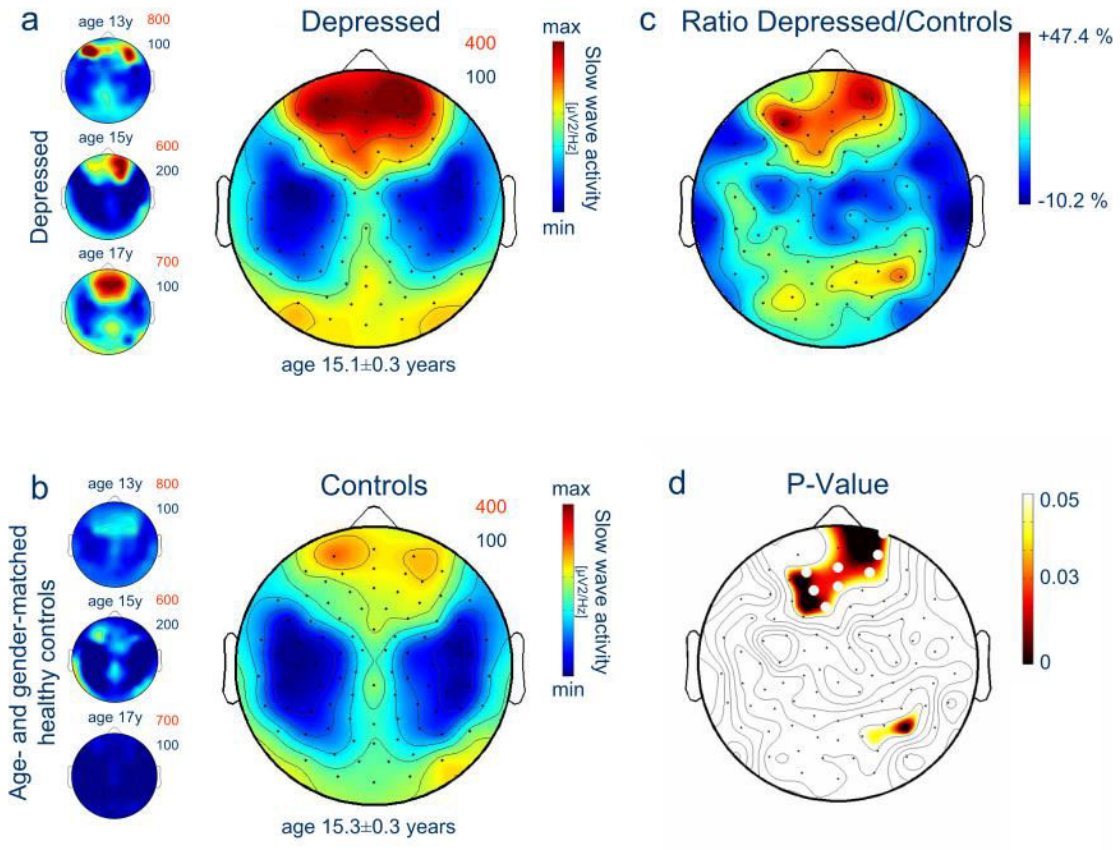

Figure 1: **a, b.** Topographical distribution of SWA (EEG power between 1-4.5 Hz) for the first hour of NREM sleep stages 2 and 3 in depressed subjects **(a)** and healthy, age- and gender-matched control subjects **(b)**. Values are colour coded (maxima in red, minima in blue) and plotted on the planar projection of the hemispheric scalp model. On the left side of each topographal plot representing the

mean of all 15 subjects, individual values of 3 participants corresponding to three different age groups (13, 15 and 17years) of the two groups (a - depressed; b - healthy controls) are presented. The numbers in the right upper corner of each topographical plot represent the maximal (red) and minimal (blue) value of SWA. **c, d.** Topographical distribution of the difference in SWA between depressed and control subjects (ratio depressed/controls). Values are colour coded (group differences in percentage %). SWA was increased by 35.2 % (±1.4% SEM) at a frontal cluster of eight significant electrodes **(c)**, indicated as white dots ($p < 0.05$, SnPM, suprathreshold cluster test controlling for multiple comparisons, Nichols and Holmes, 2002) **(d)**.

To evaluate the effects of sex, a two-way ANOVA confirmed a significant group effect with higher frontal SWA in the cluster of 8 frontal electrodes for the first hour of NREM sleep in depressed adolescents ($F(1,26) = 6.8$, $p<0.05$) but showed no effect of sex ($F(1,26) = 0.04$, $p>0.1$) and no interaction sex *x* group ($F(1,26) = 1.7$, $p>0.1$).

For an anatomical localization of the frontal cluster, orthogonal projection of the electrodes onto the cortex localized all 8 electrodes to the frontal lobe, superior frontal gyrus (7 electrodes to Brodmann area (BA) 10 and 1 electrode to BA 8).

Higher frontal SWA in depressed adolescents and their unaffected siblings remains stable across the night

We further compared the cluster of 8 frontal electrodes between unaffected siblings and age- and gender-matched controls. Our results show that compared to controls both, the depressed as well as their unaffected siblings, exhibited more SWA in the cluster of frontal electrodes (Figure 2).

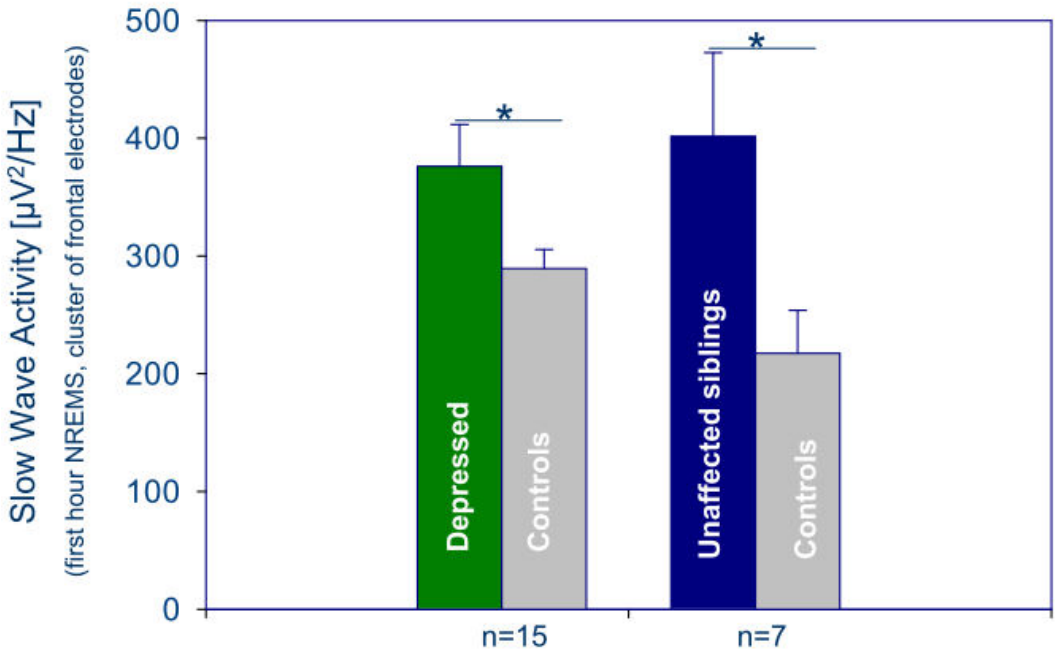

Figure 2: Between-group comparison of SWA in the cluster of electrodes showing significant differences between depressed, healthy siblings and control subjects during the first 60 min of NREM sleep stages 2 and 3. * p< 0.05 (two tailed, unpaired Student's t-test).

The dissipation of SWA across the night did not even out the regional differences between the groups. More specifically, the frontal cluster of SWA remained significantly higher in depressed and unaffected siblings compared to age- and gender-matched controls from the first to the last hour of NREM sleep. In depressed adolescents and controls a mixed model ANOVA showed a significant main effect of time (first vs. last hour of NREM sleep), $F(1,28) = 125.5$, $p<0.01$ and group, $F(1,28) = 7.4$, $p<0.05$ while there was no interaction between time *x* group $F(1,28) = 2.6$, $p>0.1$. A similar result was found when comparing the group of unaffected siblings with healthy controls (time, $F(1,12) = 34.1$, $p<0.01$; group, $F(1,12) = 5.1$, $p<0.05$; time *x* group $F(1,12) = 3.3$, $p>0.05$).

Frequency-specific frontal increase in depressed adolescents and their unaffected siblings

In order to assess whether the observed frontal increase in SWA was restricted to the SWA frequency range (<4.5 Hz), we examined the entire frequency spectrum. We found that the increase in SWA in the 8 significant frontal electrodes was specific for the low frequency range (<2Hz) in both, the depressed group (Figure 3) and the

unaffected siblings (data not shown), compared to controls. Moreover, in an exploratory analysis, we compared the patients with their siblings and found the spectral profiles to overlap, thus no differences being observed (data not shown).

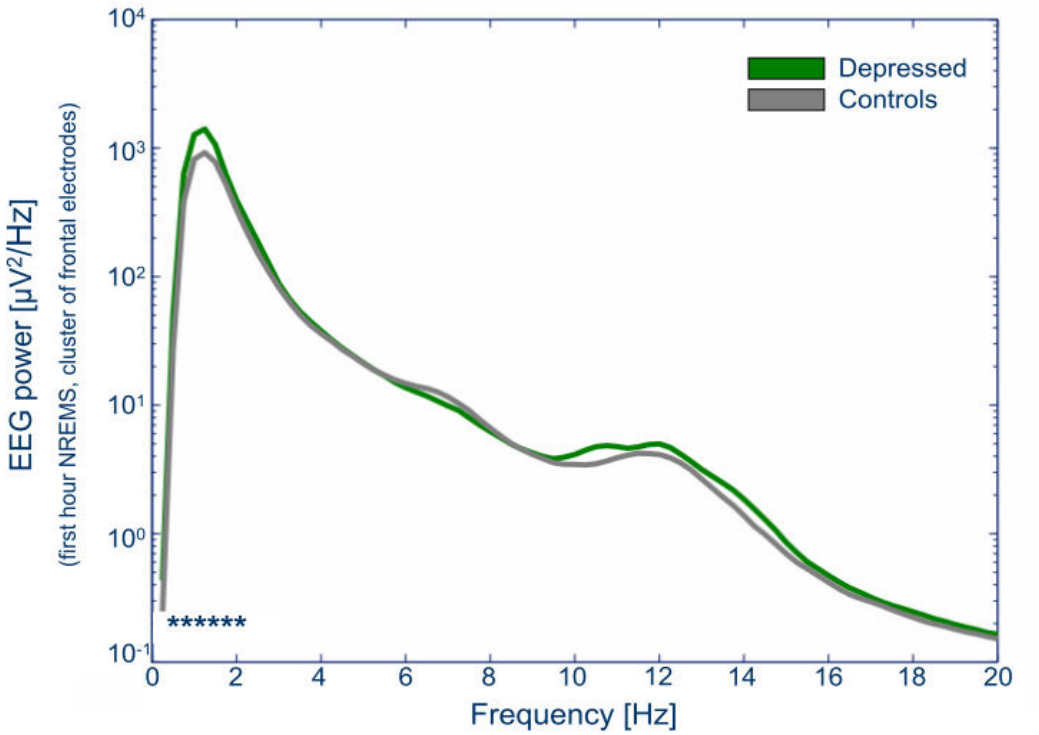

Figure 3: EEG power spectra for the cluster of eight electrodes showing significant differences in SWA between depressed and control subjects during the first 60 min of NREM sleep stages 2 and 3. * indicate frequency bins for which power in the depressed group (n=15) differed significantly ($p < 0.05$) from the controls group (two tailed, unpaired Student's t-test).

Associations with actual symptom severity

We further investigated the relationship between SWA and actual symptom severity in the affected group. Positive correlations between SWA over frontal brain regions and the CDRS-R subscore for morbid thoughts ($r=0.7$, $p=0.002$) were found. 3 of 6 electrodes showing a significant correlation were located over area BA 10 for the subscore morbid thoughts, comprising the items morbid and suicidal ideation. The total score, as well as the other four subscores such as observed and reported depressive mood, anhedonia, and somatic symptoms did not correlate significantly with SWA over frontal brain regions.

Discussion

Our study shows that the topographical distribution of sleep SWA in adolescents diagnosed with Major Depressive Disorder (MDD) shows a particular pattern, with

increased SWA over the frontal cortex compared to healthy individuals. Moreover, a similar increase in SWA was found in a group of unaffected siblings of the adolescent patients with MDD.

All groups slept equally well with a similar sleep latency and good sleep efficiency. Sleep efficiency (group means from 86 to 92%) was consistent with laboratory-based measures reported from other studies (Mason et al., 2008). Whereas changes in sleep structure in depressed adults such as reduced slow wave sleep and reduced REM latency are quite consistent (Reynolds and Kupfer, 1987), studies in early-onset depression, have yielded infrequent evidence of the same abnormalities (Tesler et al., 2013). In line with our results, several studies reported no significant group differences in any sleep variable (Dahl et al., 1991, Dahl et al., 2003, Emslie et al., 1990, Young et al., 1982). In contrast, two longitudinal studies reported altered sleep structure, such as reduced REM sleep latency and more REM sleep, to precede and co-occur in adolescents with depression (Dahl et al., 1996, Rao et al., 2002).

Regarding sleep SWA in our patient group with early onset MDD, a typical age-specific frontal predominance, sustained symmetry and bilateral temporal power minima were detected. These findings are in line with previous findings illustrating that the topography of SWA is characterized by local maxima which show an age-dependent shift from occipital areas during early childhood, central areas in late childhood to frontal areas in late adolescence (Kurth et al., 2010b). This age shift in SWA seems to be parallel to the anatomical maturation which is supported by magnetic resonance imaging studies showing that cortical development follows a similar posterior-anterior time course (Gogtay et al., 2004b, Sowell et al., 2004). Compared to healthy controls, depressed adolescents showed significantly higher values of SWA over a cluster of frontal electrodes. Specifically, the cluster of eight electrodes was localized over the frontal lobe, superior frontal gyrus (BA 8 and 10). To our knowledge, this is the first study investigating the topographical distribution of SWA in depressed adolescents and their siblings with hdEEG. A study examined at single electrodes found SWA to be lower in male adolescents compared to healthy male controls, but reported no differences in female adolescents (Lopez et al., 2012). However, from this study we do not know whether the reduced SWA in male

subjects was specific for a certain region or was present globally. A global difference might indicate a relationship to changes in sleep structure affecting SWA in a similar way at all electrodes. In line with our results a recent study (Frey et al., 2012) showed higher SWA in depressed female young adults (mean age±SEM, 22.8±3.9 years) compared to age-matched healthy controls and an older control group (mean age±SEM, 64.4±5.4 years). Even though we statistically ruled out an effect of sex on our findings, larger as well as longitudinal studies of pre- and postpubertal adolescents are needed to further disentangle the complex interaction between maturation, sex and onset of depression.

What might the local increase of SWA over frontal regions reflect? Possibly not only the depressive state, since our group of unaffected siblings showed a similar increase in SWA over frontal brain regions. This conclusion has to be drawn with caution because the number of siblings included in our study is rather low while the age-range and the inter-individual variance of SWA are large compared to the age range of the depressed adolescents. Our results correspond well to recent findings of structural imaging and the observation that SWA is positively associated with cortical thickness (Buchmann et al., 2011). Thus, a recent study showed thicker right and left middle frontal gyrus (BA 46, including portions of BA 9 and BA 10) in depressed adolescents compared to controls (Reynolds et al., 2014). Interestingly, a longitudinal study correlated anxious/depressed symptom scores with thickness of the right ventromedial prefrontal cortex and found a negative association at younger ages, a positive association from 15 years on and a shift in polarity occurring at about age 12 (Ducharme et al., 2013). Since SWA topographically peaks over areas of extensive synaptic reorganization during healthy development (Kurth et al., 2010b, Kurth et al., 2012, Wilhelm et al., 2014), the increase of frontal SWA in depressed adolescents may reflect an altered pruning of synapses in this area, such as a failure to reduce irrelevant and/or dysfunctional connections which may then result in more pronounced frontal cortical thickness (Ducharme et al., 2013, Huttenlocher, 1990, Reynolds et al., 2014). Thus, the increased frontal SWA in adolescent-onset depression and in individuals at high-genetic risk may represent an early deviant neurodevelopmental pattern.

Sleep SWA is not only reflecting maturational changes, but is use-dependently regulated on a local level. More specifically, SWA is locally increased as a result of more intense use during preceding wakefulness (Finelli et al., 2001a, Kattler et al., 1994, Huber et al., 2004). Therefore, the local increase of SWA in our sample could also reflect an increased use of the frontal cortex during the day, such as maladaptive repetitive ruminative thinking as hypothesized by Frey et al. (Frey et al., 2012). Interestingly, functional MRI studies reveal higher activation in the medial and dorsolateral prefrontal cortex and in limbic structures during rumination (Cooney et al., 2010), which might be related the frontal increase in SWA we observe in our depressed adolescents. However, we did not include a specific questionnaire for ruminative thinking, our result of a positive correlation between morbid and suicidal ideation and frontal SWA, may further support the link between ruminative thinking and locally increased SWA. The healthy siblings showing a similar frontal increase in SWA compared to control subjects did not report suicidal thoughts according to the MINI-KID, but might have used their frontal cortex more intensively for positive or neutral thinking. Against this use dependence speaks the persistence of the local difference at the end of the sleep period. Typically, use dependent increases of SWA are reduced in the course of the night reflecting the recovery function of sleep (Borbely and Achermann, 2005). This does not rule out that the observed SWA changes reflect short-term use dependent processes which might interact with long-term cortical reorganizational processes. In this regard, frequently increased daytime use of specific brain regions, during a period where major neurobiological modifications occur, could alter the time course of typical healthy cortical restructuring.

Another interesting point for discussion is related to the accumulating evidence that the neuronal sleep slow oscillations, which are reflected in the surface slow waves, may provide the ideal basis for information processing and transfer within a specific circuit (Diekelmann and Born, 2010). Thus, an “over-activation” of slow oscillations may facilitate an engraving of dysfunctional negative biased thoughts. The observed difference between objective and subjective sleep measures in our depressed adolescents may be an indication for such negative biases and altered memories (Murphy et al., 1999, Taylor Tavares et al., 2008). This observation fits well with Beck’s cognitive model of depression (Beck, 2008), which assigns biased acquisition

and procession of information a key role in the development and maintenance of depression. A puzzling question is therefore, whether sleep, i.e. sleep slow waves, are actively involved in the formation of maladaptive neuronal responses. Support for a “depressogenic” role of sleep slow waves comes from selective slow wave sleep deprivation in depressed adults which result in an amelioration of depressive symptoms (Landsness et al., 2011). However, so far selective slow wave sleep deprivation has not been investigated in adolescents with MDD.

If indeed negative biases are related to frontal SWA we might expect such a relationship also to be present in healthy participants. In an exploratory analysis, in a sample of 31 healthy adolescents and young adults (16.9±0.7 years) selected from ongoing studies in our laboratory, who had no personal and family history of psychiatric disorders, chronic diseases, learning disabilities, sleep disorders or use of medication, we correlated the difference in objective and subjective sleep latency and frontal SWA. Indeed, we found a significant positive correlation between the difference in objective an subjective sleep latency and first hour SWA in the cluster of eight frontal electrodes showing major differences in our depressed group and their healthy siblings ($r=0.5$, $p=0.007$; $r=0.3$, $p=0.08$, partial correlation, excluding the effect of age). These results support the notion that negative biases and altered memories possibly exist as part of a continuum and depend on the same neurobiological system as full blown depressive symptoms.

Taken together, SWA topography seems to be a reliable mapping tool that mirrors and/or precedes disturbed processes of cortical brain plasticity. Furthermore, SWA might represent a promising endophenotype which has the potential to identify subtle abnormalities not only in early-onset depression but already in adolescents at risk as well as in healthy individuals. By bridging the gap between structural and functional developmental alterations, our approach guides research a step closer towards the rather challenging mission to better understand and recognize early onset psychiatric diseases. The use of SWA topography as a mapping tool may offer other advantages. SWA topography directly reflects variations in the underlying spontaneous neuronal activity (Steriade et al., 2001, Timofeev et al., 2001, Vyazovskiy et al., 2009). Furthermore, the assessment of changes in neuronal activity during sleep minimizes possible confounding factors related to waking

activities, including changes in the level of attention and distractibility, and issues of motivation or cognitive capacity. The perceptual disconnection from the environment during sleep might therefore be a further advantage and particularly relevant for studies in children with psychiatric diseases.

When discussing these results we should keep in mind some limiting factors of our study. Even though we adapted our participants' bedtime to their individual habitual bedtimes, the wake up time was often slightly earlier because of net removal and other morning habits. Hereby, the total sleep time of the participants was possibly shortened which could have affected several sleep variables. Due to the naturalistic design of the study, the use of medication is present in most of the depressed adolescents, and it is unclear how these medications might have influenced SWA. However, since one third of the depressed patients were medication naive and also the unaffected, unmedicated siblings showed similar results, a direct causative influence of medication seems unlikely. The high rates of comorbidities in the depressed group reflect a rather typical clinical sample because anxiety disorders are the most frequent comorbid disorders in children and adolescents with MDD. Next to social phobia, ADHD was the most prevalent comorbidity in our sample. Interestingly, in a sample of children with the primary diagnosis of ADHD the SWA topography showed a different pattern with lower SWA in frontal areas compared to healthy controls, supporting the hypothesis of an age-dependent maturational delay in ADHD (Drechsler et al., 2005, Gustafsson et al., 2010, Kinsbourne, 1973, Ringli et al., 2013, Shaw et al., 2007). So we think that the present sample is representative and the primary diagnosis MDD was more prominent and more impairing in comparison to the comorbid disorders. On the other hand we assumed that the exclusion of anxiety disorders or ADHD would have resulted in a more atypical not representative sample. Future studies are needed to relate possible clusters of symptoms to specific topographical patterns and to confirm the specificity of the reported findings. Since depression during adolescence may also precede other mental disorders such as bipolar disorder or schizophrenia, follow-up investigations of the topographical distribution of SWA are necessary to tract deviant cortical development.

2.1.3. Individual Slow Wave Activity Trajectories as a Marker for Brain Development

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In Preparation

Abstract

Background

Recent evidence proposes that the sleep EEG presents a "window through which adolescent brain development can be viewed". Particularly sleep slow wave activity (SWA) matches the trajectory of cortical development. SWA, a major electrophysiological rhythm between 0.75-4.5 Hz of non-rapid eye movement (NREM) sleep is a well established marker of sleep homeostasis and sleep depth. SWA changes profoundly during the first two decades of life presumably reflecting the restructuring in synaptic number and efficiency. Using high-density EEG (hdEEG) (128 electrodes) in a cross-sectional design, it has been demonstrated that the region expressing maximal SWA relative to other brain regions shifts from posterior to frontal regions throughout development. This frontalization of SWA has not been investigated in a longitudinal setting and is therefore an important goal of this study.

Methods

Therefore, we aimed to characterize by means of hdEEG the topographical aspects of SWA that remain stable during adolescence and aspects that change within and between subjects across this time period. Furthermore, we investigated the individual changes of SWA during adolescence in relation to behavioural changes in a specific visuomotor task.

Results

Our data convincingly shows that relative SWA topography is a strong trait but also provides coherent changes in central and frontal derivations that can be tracked within and across individuals. In addition, development of relative SWA in central regions could be related to the stability or inter-joint coordination in a goal directed reaching task.

Conclusions

In conclusion, our findings support that the sleep EEG, in particular SWA, is a strong trait which can be used as a mirror for (motor) skill development and cortical restructuring during adolescence.

Introduction

The brain faces pronounced cortical restructuring during adolescence that is marked by a pruning of synapses. This process does not happen in all cortices at the same time but starts in primary cortices, moves forward and outward and ends in the prefrontal cortex (Gogtay et al., 2004a, Shaw et al., 2008) structural mapping of those brain developmental changes can be obtained by magnetic resonance imaging (MRI). Recent evidence proposes that the sleep EEG provides a functional correlate of this pruning process and presents a "window through which adolescent brain development can be viewed" (Colrain and Baker, 2011b, Tarokh et al., 2011). Epecially sleep slow wave activity (SWA) matches the trajectory of cortical development (Ringli and Huber, 2011). SWA is a major electrophysiological rhythm between 0.75-4.5 Hz of non-rapid eye movement (NREM) sleep and a well established marker of sleep homeostasis and sleep depth (Achermann and Borbely, 2011). Slow waves are related to the slow oscillations that rest on the low-frequency oscillation (1 Hz) in the membrane potential of cortical neurons (Amzica and Steriade, 1998, Contreras and Steriade, 1995, Steriade et al., 1993b). The synchronization of the slow oscillation of many neurons lead to the characteristic slow waves measured in the surface EEG. SWA likely mirrors synaptic density because more and stronger synapses benefit synchronization (Esser et al., 2007, Vyazovskiy et al., 2009). SWA changes profoundly during the first two decades of life presumably reflecting the restructuring in synaptic number and efficiency (Ringli and Huber, 2011). Indeed, longitudinal and cross-sectional studies in humans show that SWA follows the time course of an inverted U-shape with a peak shortly before puberty similar to the time course of synaptic density (Campbell and Feinberg, 2009, Feinberg, 1982, Feinberg et al., 2011, Zuo et al., 2005). A recent study supports the association between EEG derived SWA and grey matter volume assessed with MRI in different cortical regions in subjects between 4 and 19 years of age (Buchmann et al., 2011). A few studies further demonstrated that SWA development shows regional differences (Feinberg et al., 2011, Jenni et al., 2005, Kurth et al., 2010b) as does the thinning of the cortex (reflecting synaptic pruning) measured in cross-sectional and longitudinal MRI studies (Gogtay et al., 2004a, Shaw et al., 2008). In a longitudinal setting of sleep EEG recordings, (Feinberg et al., 2011) reported that the SWA decline shows regional differences and follows a

back (occipital electrode) to front (frontal electrode) maturational pattern. However their topographical analysis was limited to 5 referential electrodes. Using high-density EEG (128 electrodes) in a cross-sectional design, (Kurth et al., 2010b) demonstrated that the region expressing maximal SWA relative to other brain regions shifts from posterior to frontal in 2-25 years old subjects, reaching frontal derivations during adolescence and therefore parallels cortical brain development. This frontalization of SWA has not been investigated in a longitudinal setting and is therefore an important goal of this study.

During adulthood SWA shows a typical topography with a prefrontal maximum that remains stable individually (trait) but varies profoundly between subjects (Finelli et al., 2001a, Finelli et al., 2001b). Since we have a marked decrease of SWA of about 60% between 11 and 16 years (Campbell and Feinberg, 2009, Feinberg et al., 2006) it could be argued that during adolescent brain development this intraindividual stability is not true. However, (Tarokh et al., 2011) recently demonstrated that trait-like aspects in the sleep EEG spectra exist across adolescence despite considerable cortical changes. We further aimed to characterize the topographical aspects of SWA that remain stable during adolescence and aspects that change within and between subjects across this time period.

Finally, there is good evidence that SWA is a reliable indicator of synapse number and efficiency, and synaptic specialization in turn is needed for the development of specific functions (e.g. motor skills). Thus, SWA changes should be related to or even involved in the maturation of specific skills. (Kurth et al., 2012) found that the maturity of the slow wave topography was related to complex motor task performance. Furthermore, correlations between motor performance of visuomotor learning and SWA activity in an adult population were reported (Huber et al., 2006, Huber et al., 2004). We further investigated the individual changes of SWA during adolescence in relation to behavioural changes in a specific visuomotor task.

Methods

Subjects

6 subjects (aged between 9.4 and 13.1 years, 3 females), selected from a larger dataset published in (Kurth et al., 2010b), participated in follow-up measurements. All of them underwent a telephone and questionnaire screening to exclude subjects with sleep disorders, chronic diseases, personal or familial history of psychopathology, current use of psychoactive agents or medications and left-handedness. Furthermore, none of the subjects travelled across more than one time-zone in the 4 months before the study. The procedure was approved by the cantonal ethics commission in Zurich, and the study was performed according to the declaration of Helsinki. Parents or subjects of full age gave written informed consent after being explained the study procedures and aims in detail.

To ensure stable conditions subjects were required to maintain a regular sleep schedule one week prior to measurements. Actigraphy and sleep diaries (kept by participants or parents) were used to verify compliance. Neither napping, caffeine consumption, medication nor alcohol consumption were allowed 24-h prior to the sleep recordings. Since sleep rhythms vary across the menstrual cycle and show lowest variability in the follicular phase (Driver et al., 1996), we measured post-pubertal female subjects during this phase.

Procedure

All subjects had 3 experimental nights (baseline, follow-up night 1 [FU1], follow-up night 2 [FU2]) in total with 2.6 ± 0.5 years and 2.5 ± 0.1 years in between, respectively. The procedure was exactly the same for all 3 nights including a visuomotor learning task in the evening and all-night sleep EEG recordings. Individually reported bed times were used and subjects were awakened in the morning to allow school and job participation resulting in variable sleep lengths.

Sleep EEG recordings and analysis

A high-density EEG (hdEEG) cap with 128 electrodes (including EOG) was adjusted to the Vertex (Cz) and filled with gel electrolyte. In addition, two submental EMG electrodes were applied. Besides high temporal resolution, the hdEEG provides good spatial resolution allowing the analysis of topographical and

local aspects of sleep (Lustenberger and Huber, 2012). The EEG, EOG and EMG were constantly recorded during the whole sleep period. The signals were rereferenced to Cz and digitized at 500Hz (band-pass filtering 0.01-200Hz). Afterwards the signal was offline high-pass filtered at 0.5 Hz, low-pass filtered at 50 Hz and downsampled to 128 Hz. Sleep data was visually scored in 20 s epochs according to the American Academy of Sleep Medicine standard criteria (Iber, 2007). Artifact removal was done visually and if power exceeded a threshold based on a sliding mean of power in the 0.75-4.5 Hz or 20-30 Hz range (Huber et al., 2000).

EEG spectral power of consecutive 20-s epoch was calculated using a Fast Fourrier Routine (Hanning window, average of five 4 s windows, frequency resolution 0.25 Hz). The spectral analysis was performed for the first hour of artefact free NREM sleep. We selected this interval because (1) it was used by (Kurth et al., 2010b) to map SWA topography in the first two decades of life in a cross-sectional design (2) it belongs to the most consolidated part of sleep (3) it includes the same number of epochs for all subjects and (4) some subjects had many bad quality EEG electrode recordings towards the end of the sleep period. Spectral analysis was performed for all marginal electrodes (109) which allowed us topographical mapping of the data. EEG data was average referenced only including good quality channels and bad quality channels (on average 2.4 ± 2.3 per night) were interpolated using a spherical interpolation provided by EEGLAB toolbox (Delorme and Makeig, 2004). EEG Power values for each electrode within a map were normalized (Kurth et al., 2012). We used this normalization because we were interested in relative regional changes rather than the gross changes in signal amplitude that occur in all derivations during adolescence. Sleep EEG frequency bands were defined according to (Kurth et al., 2010b). We mainly focussed on SWA (1-4.5 Hz). For additional analysis we further calculated theta activity (4.75-7.75 Hz), alpha activity (8-9.75 Hz), sigma activity (10-15 Hz) and gamma activity (20-25 Hz).

We further analysed the number of subjects that followed a specific trajectory in how relative SWA changed (steady increase or steady decrease) across the 3 measurements. This analysis was performed for all electrodes. We then defined

the maximal cluster of electrodes/electrode (regions of interest; ROIs) that followed the same trajectory in at least 5 subjects. We further defined for these specific ROIs how many subjects followed a similar trajectory for the other frequency bands.

Motor task

In the evening a visuomotor target reaching task was performed. Therefore, subjects moved a cursor with their right hand on a digitizing table to one of four targets displayed on a screen. Targets were separated by 45° and distance was kept by 8 cm to a common starting point. Targets were presented every second in a pseudo-randomized order. Subjects were instructed to reach them and go back to the starting point within this second in one straightforward movement as precise and smooth as possible and with no breaks in between. They first performed two blocks (including 44 movements each) to familiarize them with the testing device and the movement. The third block was used for the analysis. Different variables can be obtained from this task. To avoid multiple comparisons we focussed on two specific variables that were used in prior studies in a slightly different form and task design, and have been related to SWA changes (Huber et al., 2006, Huber et al., 2004). Thus we focussed on mean absolute Directional Error, which measures the angular difference between the direction of the target from the initial hand position and the direction of the hand at the peak outward velocity from the same initial point. We further investigated the absolute Normalized Movement Area that represents the area enclosed by the hand-path divided by the squared path length. We then computed the standard deviation of this area between the movements of the block (variability, a measure that might be closely related to inter-joint coordination (Huber et al., 2006, Krakauer et al., 1999). Movements to the wrong target or clearly deviant from the target (Directional Error > 2.5 * SD of the mean), false starts and movements with breaks in between were excluded.

Statistical analysis

We used R statistical software for all analysis. We first pooled the data of the three measurements (3 observations per subject, 18 observations in total) to relate normalized SWA of the selected ROIs to the motor task variables. We further controlled for age, number of excluded trials and movement onset time in a partial

correlation design using Pearson correlation. We repeated the same analysis but used the differences between baseline measurement and FU1, and FU1 to FU2, resulting in 12 observations (2 differences per subject). To exclude the possibility that repeated observations per subject drive significant correlations, we performed a linear model of the residuals and included subjects as a factor to test whether repeated observations significantly contribute to the model. In case the factor "subjects" significantly affected the model, we additionally performed a linear mixed model and related the residuals to each other but included subject as a random factor in the model. P-values < 0.05 were considered significant.

Results

Trait-like aspects, intra- and interindividual changes of SWA topography

The topography of relative SWA for each subject and night is plotted in Figure 1. The topographical distribution varies substantially across individuals but is rather stable within one subject even though years are between the measurements. Thus, we have a clear trait in the SWA topography. Nevertheless, we also see within subject changes with an increase in frontal areas (frontalization) and decrease in central areas. Of note, for a specific prefrontal electrode all subjects increase steadily their relative SWA in the course of the 3 measurements (Figure 2). For a specific central cluster a constant decrease can be observed in all subjects. Also theta and alpha power are steadily declining in this central electrode cluster in more than half of the subject, whereas in the frontal electrode, the increase is restricted to the SWA range (Figure 3).

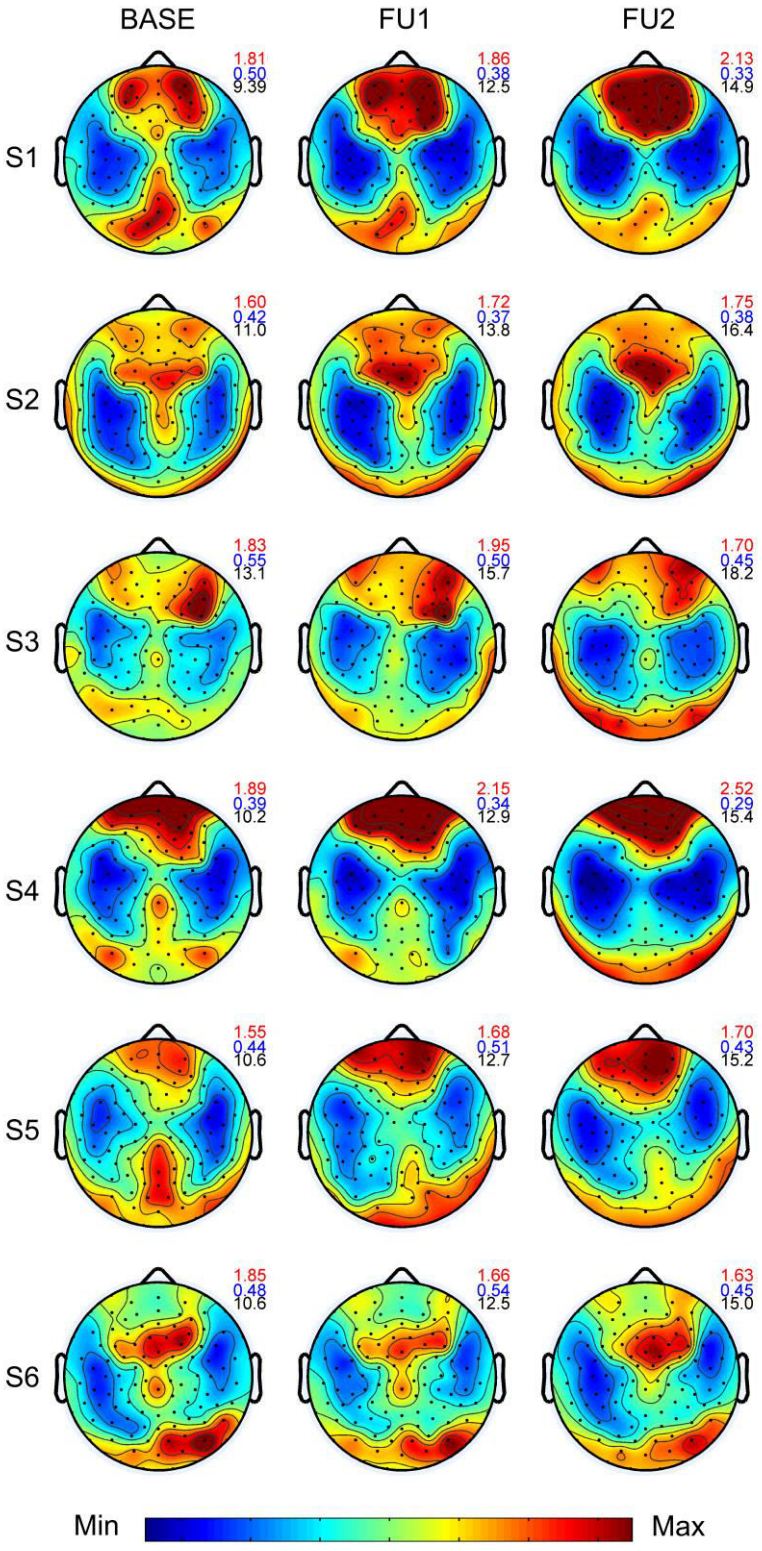


Figure 1: Topographical distribution of relative SWA (EEG Power 1-4.5Hz) of the first hour of NREM sleep for 6 individuals (rows) and 3 nights (column) each. Hot colors denote maximal relative SWA and cold colors minimal activity. In the top right of the maps, number indicates maximal (red) and minimal (blue) relative SWA, and the age of the subject (black).

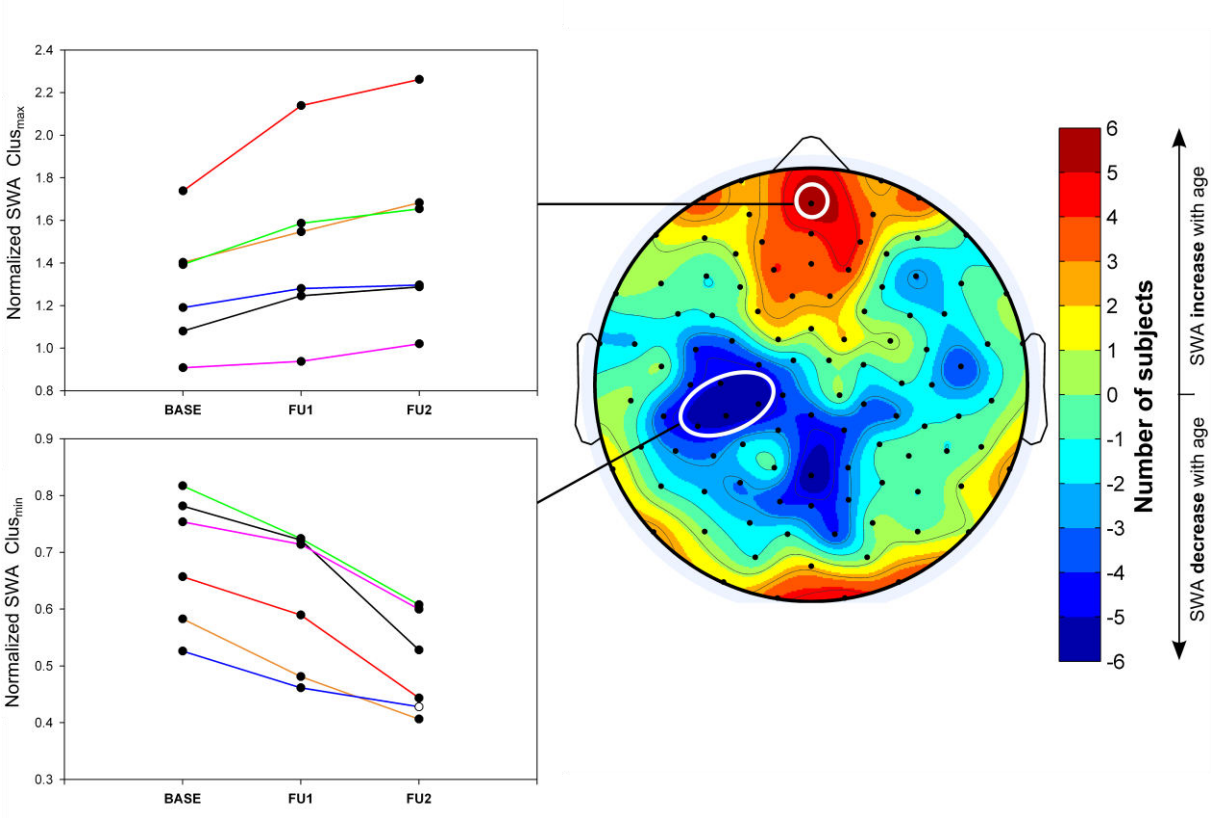


Figure 2: Individual SWA trajectories for two specific regions of interest (ROIs) and all 3 measurements. The maximal number of electrodes with a steady increase (frontal electrode) or decrease (central cluster) in more than 5 subjects (topographical plot) was defined a ROI. Subjects in the trajectory plots are color coded (also used in Figure 4). FU1: 1st follow-up measurement; FU2: 2nd follow-up measurement.

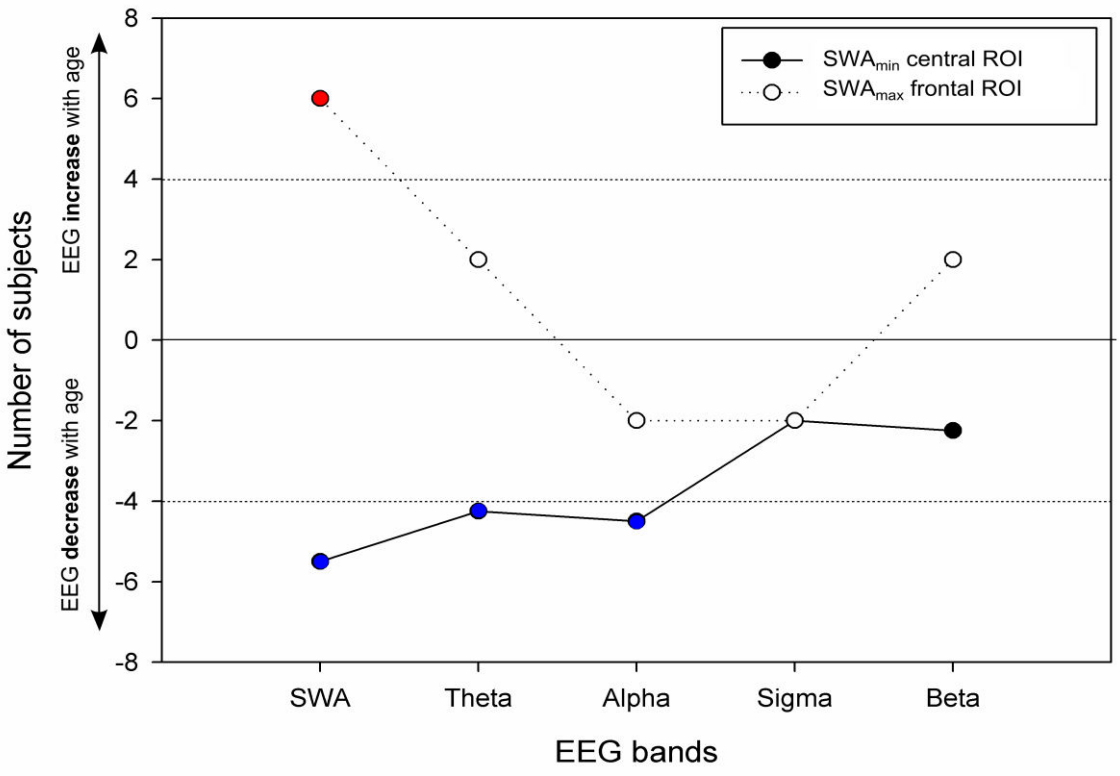
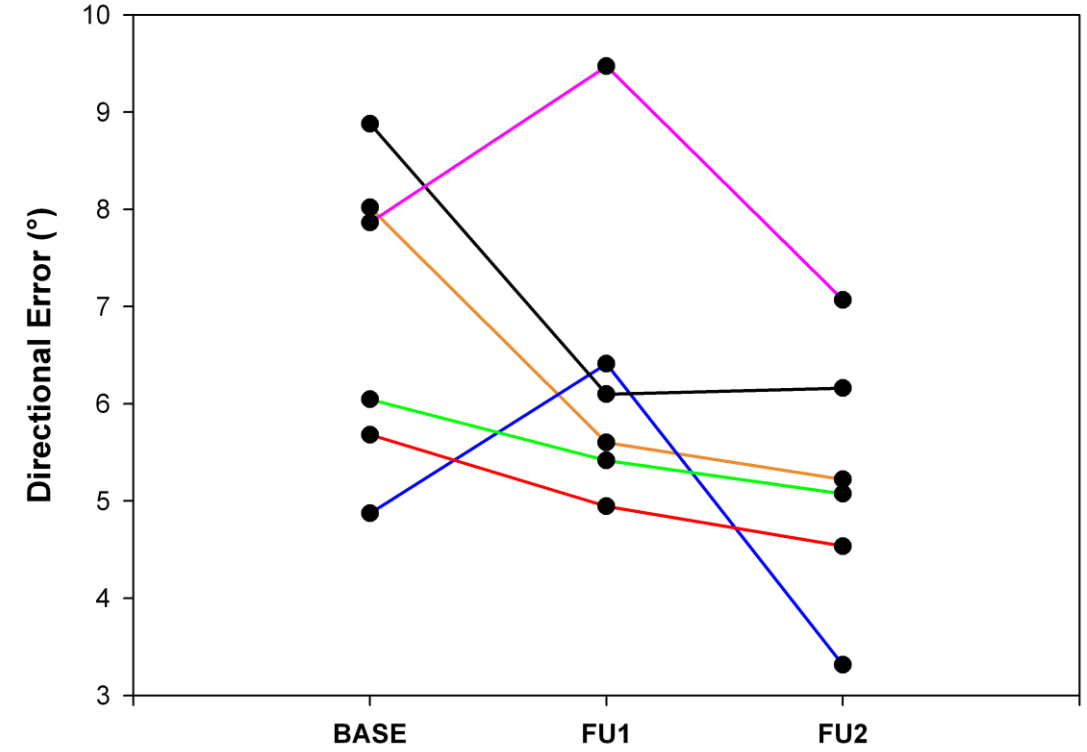


Figure 3: Number of subjects with a steady increase or decrease for different frequency bands for a frontal and central ROI (q.v. Figure 2). If more than half of the subject (>3) had a similar trajectory they were highlighted in red and blue, respectively.

SWA changes and visuomotor behaviour

Recent evidence exist that the topographical distribution of SWA predicts cortical development and the maturation of specific skills (Buchmann et al., 2011, Kurth et al., 2012). We further investigated whether the relative SWA and SWA changes across the measurements in our selected ROIs are related to behavioural measures of a visuomotor reaching task. Correlation coefficients and p-values are listed in Table 1. Figure 4 illustrates the individual trajectories of the Directional Error and the variability of the Normalized Movement Area. We found a significant positive correlation between relative SWA in the central ROI with the variability of the Normalized Movement Area (Figure 5A). Also the differences between the measurements of these two variables were positively related (Figure 5B). Variability of the Normalized Movement Area was not correlated with the frontal ROI. Mean Directional Error was negatively associated with the relative SWA of the frontal ROI, but no significant relation was found for the differences. These correlations also persisted after controlling for repeated measures (see methods). Furthermore no association was observed between Directional Error and relative SWA of the central cluster, neither for the mean nor for the differences. Finally,

some significant correlations between the behavioural measures and SWA of the specific ROIs were also found for other frequency bands.



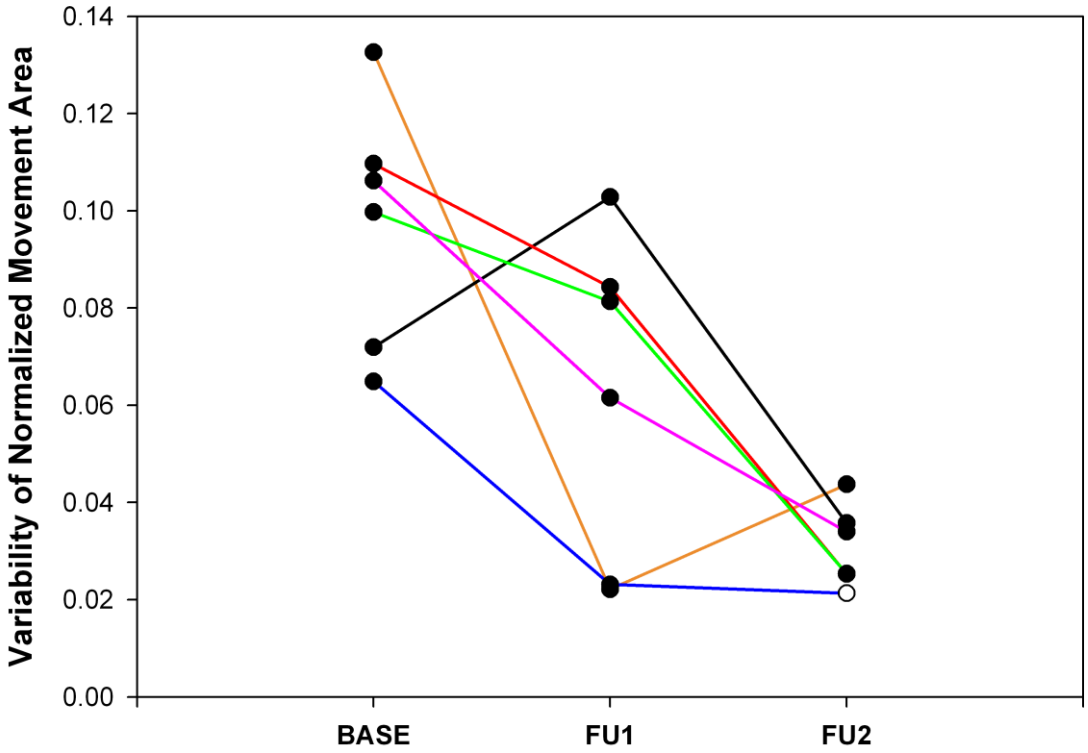


Figure 4: Individual visuomotor performance trajectories in the course of the 3 measurements. Upper panel illustrates directional error and lower panel variability (standard deviation) of the normalized movement area. Subjects are color coded (q.v. Figure 2).

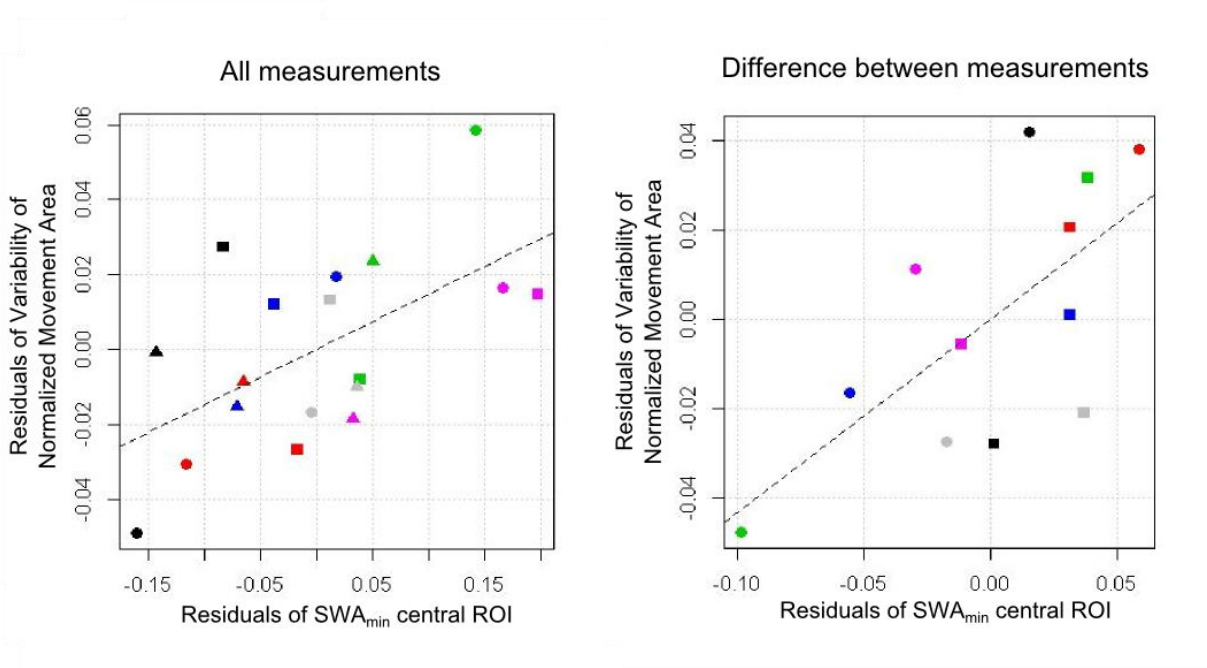


Figure 5: Correlations between residuals (partial correlation design, controlled for age, number of included trials and movement onset time) of SWA of the central ROI and residuals of the variability of Normalized Movement Area for A all measurements and B the differences between measurements. Similar colors belong to the same individual.

Discussion

SWA provides a functional correlate of cortical reorganization and skill maturation, and undergoes prominent changes until it reaches mature stages during adulthood. In this study we investigated SWA topography across adolescent development in a unique longitudinal study setting to assess its trait-like aspects, and within and between subject changes. We then investigated the relationship between SWA of specific ROIs and visuomotor target reaching skills.

Trait-like aspects, intra- and inter-individual changes of SWA topography

Even though adolescence is marked by prominent cortical restructuring and SWA changes, we found a strikingly stable SWA topography in our study. This finding is intriguing since (1) all subjects show a fingerprint like topography but vary profoundly between each other and (2) several years are between the

measurements. Using a central and occipital EEG derivation (Tarokh et al., 2011) reported that morphology of the sleep EEG spectrum is largely preserved between repeated measures (years in between) in adolescent subjects with SWA showing infraclass correlation coefficients (ICC, measure of stability) as high as those observed in adults. We add an important dimension to their findings and show that also SWA topography is highly preserved across adolescence. It is important to mention that we used normalized (to all electrodes) SWA values since absolute power would show a very pronounced decrease in all electrodes. However, we were especially interested in regional differences rather than the high reduction in power. A normalization (to the whole spectrum) was also used by (Tarokh et al., 2011) to allow the investigation of morphological differences rather than to highlight the pronounced reduction in power. How can these trait-like aspects be explained? The sleep EEG seems to be highly determined by the genetic background for which evidence is provided in twin studies (De Gennaro et al., 2008, Landolt, 2011). Intriguingly, SWA topography represents also good biological endophenotype even in periods of pronounced cortical restructuring (Tarokh et al., 2011) which gives a hint that Sleep EEG in general and SWA topography in particular is among the strongest heritable traits in humans.

Besides trait-like aspects, the topographical distribution of SWA also showed steadily within-subject changes across the years. When visually inspecting Figure 1, we clearly see that the relative amount of SWA increases in frontal regions and decreases in central regions. This finding is consistent between subjects as was revealed in our trajectory analysis illustrated in Figure 2. For a specific prefrontal electrode all subjects steadily increased the relative SWA across adolescence. Also in a large cluster of surrounding electrodes 4 of 6 subjects showed this steady increase. This increased frontalization with age has earlier been reported in a cross-sectional study design showing a SWA peak shift from posterior to anterior regions in 2-25 year old subjects (Kurth et al., 2010b), which is now strongly supported by our longitudinal findings. Of note, in only one electrode all subjects showed a constant increase in SWA. This could be explained by the observation that even though all subjects seem to increase SWA in a broader frontal region, this region is very variable between subjects. A more consistent decrease across all subjects was found in a left central cluster. This cluster is roughly located over

Brodmann Areas (BA) 3, 4 and 6 (defined using MRI based anatomical electrode location of n = 40 subjects (Kurth et al., 2010b, Kurth et al., 2012). Those BAs include the primary sensory and motor cortex, and premotor and supplementary motor areas and are therefore important for simple and complex motor skills (Kurth et al., 2012). In contrast to the frontal region, this ROI and its prominent decline across adolescence was not subject of discussion in earlier studies that focussed on development and SWA. Such a decline might highlight ongoing synaptic pruning which is related to a thinning of the cortex and therefore leads to less pronounced SWA. A possible explanation why this central ROI is restricted to the left side might be that all subjects were right handed and are therefore more using their left hemisphere to perform arm motor tasks, possibly leading to a more pronounced or faster specialization of this region. Further studies are needed to compare these findings in a left-handed study population. Finally, we investigated whether these steady changes in the two ROIs are restricted to the SWA range or also found in other frequency bands. The steady increase in the frontal ROI was clearly restricted to the SWA range whereas for the central cluster more than half of subject showed a steady decrease also in the theta and alpha frequency bands.

SWA changes and visuomotor target reaching behaviour

Ongoing maturational changes in performance of motor skills (e.g. fine-tuned motor movements, sequential finger movements, inter-joint coordination) tasks can be seen from childhood to adulthood. Evidence for an association between the maturation of SWA and complex motor skills (Kurth et al., 2012) as well as between SWA changes and motor performance in specific visuomotor tasks exist (Huber et al., 2006, Huber et al., 2004). Since our study population showed progressive alterations in SWA for a specific frontal and central ROI we were interested in how those alterations are associated with the performance in a visuomotor reaching task. Goal-directed, accurate reaching is an action used in every day's activities and shows that stability of reaching improve in 4 to 11 years old subjects (Schneiberg et al., 2002) and during adolescence (Choe, 2009). In our sample we further see a progressive decrease of the variability of the Normalized Movement Area during target reaching in 5 of 6 subjects. Relative SWA in the central ROI were predictive for the variability in the Normalized Movement Area, with lower SWA values related to lower variability. Furthermore, the more

pronounced SWA decreased across adolescence the more improvement (reduction) in variability of the Normalized Movement Area was found, a measure that presumably reflects inter-joint coordination (Huber et al., 2006). We can therefore argue that lower values in this central ROI might be related to a more mature cortical region, more efficient and specialized synapses and therefore a more stable reaching movement. As mentioned before this central cluster is located over sensorimotor areas and is therefore important for a variety of motor skills, e.g. inter-joint coordination. However, interpretations of this region should be done cautiously since we cannot directly relate the cortical source of SWA to EEG topography (inverse problem). Of note, these relationships cannot be explained by age, number of included trials, movement onset time or repeated measurements per subject, since we carefully controlled for those factors in a partial correlation design or linear mixed model. Finally, these associations were not restricted to the SWA but also found in all other frequency bands, especially for the relative SWA values but less so for the difference between the measurements (q.v. Table 1). The central cluster was not related to the Directional Error that measures the accuracy of the reaching movement. In contrast we found a significant negative correlation between relative SWA in the frontal ROI with Directional error, but not for the difference between the measurements. Furthermore, no correlation was found between the Variability of the Normalized Movement Area and SWA in the frontal ROI. Thus the progressive frontalization (increase over time) is not related to the assessed motor skills. Better behavioural measures that are more likely related to the maturation of the selected frontal ROI are cognitive skills (e.g. executive functions) that are mainly controlled by the frontal cortex (Stuss, 2011).

Limitations and outlook

Our results should be considered in the context of a small number of subjects which gives us low statistical power. However, several findings are consistent for all subjects and are therefore very convincing. A further limitation is our gap to relate the SWA measures and changes over time to direct measures of cortical maturation (e.g. grey matter volume). During our first measurements we also obtained structural T1 MRI images and calculated grey matter volumes for specific regions using Freesurfer version stable v4.5.0 for Mac OS 10.5.2 (http://surfer.nmr.mgh.harvard.edu; see also (Dale et al., 1999, Fischl et al., 1999).

Since we have only 6 subjects (and only 1 observation each), statistical analysis is very weak. Nevertheless, we found a significant positive correlation between the mean volume of the precentral, postcentral and middle frontal gyrus (located beneath our EEG defined central ROI) and SWA in this region (Pearson correlation, $r = 0.87$, $p = 0.025$, $n = 6$, age was not significantly correlated with SWA or cortical volume and therefore not included as a covariate). This finding gives an initial hint that our SWA of the central ROI is directly related to cortical volume. No correlation between the SWA of the frontal ROI and frontal cortex volume was found, however due to low number of subjects and only one electrode contributing to the ROI this finding should not be weighted too much. Future studies are needed that simultaneously record sleep EEG changes and MRI measures across adolescence in a longitudinal setting. Finally, our findings should also be investigated in the context of clinical populations. In particular, individuals with attention-deficit hyperactivity disorder (ADHD) could be investigated as they show a significant increase of SWA compared to controls in a central cluster of electrodes that overlap with our specific central ROI (Ringli et al., 2013).

Conclusion

Collectively, our data convincingly shows that relative SWA topography is a strong trait but also provides coherent changes in central and frontal derivations that can be tracked within and across individuals. Furthermore, development of relative SWA in central regions could be related to the stability or inter-joint coordination in a goal directed reaching task. Previous literature proposes a tight relationship between SWA and cortical maturation. Thus, our findings further support that the functional sleep EEG, in particular SWA, can be used as a mirror for (motor) skill development and cortical restructuring during adolescence.

2.2. Research Part 2:

Sleep and plasticity in the context of natural environmental influences – moderate altitude

2.2.1. Are Nocturnal Breathing, Sleep, and Cognitive Performance Impaired at Moderate Altitude (1630-2590m)?

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Abstract

Study Objectives

Newcomers at high altitude (>3000m) experience periodic breathing, sleep disturbances, and impaired cognitive performance. Whether similar adverse effects occur at lower elevations is uncertain although numerous lowlanders travel to moderate altitude for professional or recreational activities. We evaluated the hypothesis that nocturnal breathing, sleep and cognitive performance of lowlanders are impaired at moderate altitude.

Design

Randomized cross-over trial.

Setting

University hospital at 490m, Swiss mountain villages at 1630m and 2590m.

Participants

51 healthy men, median (quartiles) age 24y (20-28), living below 800m.

Interventions

Studies at Zurich (490m) and during 4 consecutive days at 1630m and 2590m, respectively, 2 days each. The order of altitude exposure was randomized. Polysomnography, psychomotor vigilance tests (PVT), the number back test, several other tests of cognitive performance, and questionnaires were evaluated.

Measurements and Results

The median (quartiles) apnea/hypopnea index at 490 m was 4.6/h (2.3;7.9), values at 1630 and 2590m, day 1 and 2, respectively, were 7.0/h (4.1;12.6), 5.4/h (3.5;10.5), 13.1/h (6.7;32.1), 8.0/h (4.4;23.1); corresponding values of mean nocturnal oxygen saturation were 96% (95;96), 94% (93;95), 94%(93;95), 90%(89;91), 91%(90;92), $P<0.05$ vs. 490m, all instances. Slow wave sleep in the first night at 2590m was 21% (18;25) vs. 24%(20;27) at 490m ($P<0.05$). Psychomotor vigilance and various other measures of cognitive performance did not change significantly.

Conclusions

Healthy men acutely exposed during 4 days to hypoxemia at 1630m and 2590m reveal a considerable amount of periodic breathing, and sleep disturbances. However, no significant effects on psychomotor reaction speed or cognitive performance were observed.

Introduction

Uncontrolled observations suggest that sleep in unacclimatized newcomers to high altitude (>3000 m) is unrefreshing and disturbed by high altitude periodic breathing related to an enhanced loop gain of respiratory control (Khoo et al., 1982, Nussbaumer-Ochsner et al., 2012b). Manifestations of altitude related illness including headache and malaise, and potential direct adverse effects of hypoxia may additionally affect sleep quality (Burgess et al., 2004, Johnson et al., 2010, Nussbaumer-Ochsner et al., 2012c, Reite et al., 1975, Salvaggio et al., 1998). Some studies have also suggested impairments in cognitive performance and mood at simulated or real altitude >3000 m (de Aquino Lemos et al., 2012, Virues-Ortega et al., 2004). While climbing to such elevations is generally performed by only a few well trained subjects, the number of persons who travel to settlements and tourist destinations at more moderate altitudes of 1000 to 3000 m is very large (Huddleston B. et al., 2003). According to the World Tourism Organization, mountain tourism accounts for 15-20 % of worldwide tourism, i.e., it involved 147.5 to 196.6 million people in 2011 (Organization, 2012, Programme, 2009). Nevertheless, it has not been conclusively studied whether healthy mountain travelers experience nocturnal breathing and sleep disturbances already at moderate altitude (<3000 m) and whether this is associated with cognitive impairments during daytime. Recently, we observed that patients with untreated obstructive sleep apnea syndrome living below 800 m revealed a major exacerbation of sleep apnea, pronounced hypoxemia and impaired driving simulator performance during a stay in the mountains at 1860 m and at 2590 m (Nussbaumer-Ochsner et al., 2012a, Nussbaumer-Ochsner et al., 2010). If similarly striking effects would occur even in healthy individuals without preexisting breathing disorder this would have major implications for a large number of travelers to moderate altitude as well as for flight passengers and air crew (Muhm et al., 2007, Muhm et al., 2009) in terms of impairment of subjective well-being, and performance during professional activities and sports. Therefore, the purpose of the current study was to perform a randomized trial evaluating the hypothesis that acute exposure to hypobaric hypoxia at moderate altitude during 4 days is associated with periodic breathing, sleep disturbances and impaired psychomotor and cognitive function during daytime.

Methods

Study design and setting

This randomized cross-over trial evaluating effects of moderate altitude on breathing, sleep and psychomotor performance was carried out from July to October 2010. Baseline evaluations were performed during 1 day at the University Hospital of Zurich (490 m, 1608 ft, barometric pressure [PB] 719 Torr, baseline), altitude studies were performed during 4 days in Swiss alpine villages, 2 days at Davos Wolfgang (1630 m, 5348 ft, PB 630 Torr; 3) and 2 days at Davos Jakobshorn (2590m, 8497 ft, PB 562 Torr). At all locations, sleep studies and daytime evaluations were performed in quiet single-rooms.

Participants

Healthy men living below 800 m, 18 to 70 y old, with a body mass index of 18 to 30 kg/m^2 were invited to participate. Smokers, persons with regular use of alcohol, drugs, on regular medication or with previous intolerance of moderate altitude (<3000 m) were not admitted. The study was approved by the Cantonal Ethics Committee, subjects gave written informed consent.

Randomization and interventions

Participants were randomized to 4 groups with permuted sequences of altitude exposure according to a balanced block design: group A) 490 m/1630 m/1630 m/2590 m/2590 m, B) 490 m/2590 m/2590 m/1630 m/1630 m, C) 1630 m/1630 m/2590 m/2590 m/490 m, D) 2590 m/2590 m/1630 m/1630 m/490 m. Randomization was performed by letting subjects register for one of the available study time slots according to preference and availability without being aware of the corresponding altitude exposure sequence. Subjects were instructed to sleep regularly for >7 h per night in the week preceding the study. Compliance with this requirement was verified by actigraphy. Participants travelled by train between Zurich and Davos Wolfgang and by cable car between Davos train station and Davos Jakobshorn. The trial ended as planned. Subjects were busy most of the day with various tests and they remained within the range of the hotel area except for transfers as part of the protocol. They had to avoid strenuous exercise. Napping

was not allowed and this was regularly checked by the investigators. Three meals were served per day on a regular schedule. Coffeinated drinks were not allowed.

Measurements and outcomes

Sleep studies

Polysomnography was performed from 23:00 h to 6:00 h (Alice 5, Philips Respironics AG, Zofingen, Switzerland) as previously described (Nussbaumer-Ochsner et al., 2010). Recordings included electroencephalogram, electro-oculogram, submental and bilateral anterior tibial electromyogram, pulse oximetry, capnography of expired air, calibrated respiratory inductive plethysmography, nasal prong pressure recordings (Thurnheer et al., 2001), and bilateral diaphragmatic surface EMG (Maarsingh et al., 2000). Sleep stages and arousals (Rechtschaffen, 1968), respiratory events and breathing pattern characteristics (Bloch et al., 1997) were determined as described previously (Bloch et al., 2010, Latshang et al., 2012, Nussbaumer-Ochsner et al., 2012c) by investigators blinded to the clinical data and study location. Apneas/hypopneas were defined as a reduction in the inductive plethysmographic sum signal or the nasal pressure swings to <50% of the preceding 2 min baseline during ≥10 s. Transient reductions in breathing amplitude to <50% baseline for 5-10 s were also scored as apneas/hypopneas if they occurred as part of a periodic breathing pattern with central apneas/hypopneas for >3 consecutive cycles (Bloch et al., 2010, Latshang et al., 2012, Nussbaumer-Ochsner et al., 2012c). The apnea/hypopnea index and the oxygen desaturation index (ODI, >3% dips) were computed as number of events per hour of sleep.

Vigilance, psychomotor and cognitive performance

Tests were performed between 10:00-12:00. Assessments included the psychomotor vigilance test (PVT) (Basner and Dinges, 2011), the divided attention steering simulator (DASS) (Juniper et al., 2000), the 1-, 2-, 3-back task (Regel et al., 2007) and the trail making test A (Tombaugh, 2004).

Clinical examination and questionnaires

After waking up in the morning, acute mountain sickness (AMS) was assessed by the Environmental Symptoms Questionnaire cerebral score (AMSc) (Sampson et

al., 1983) with values >0.7 considered clinically relevant AMS (Maggiorini et al., 1998). Subjective sleep quality was rated on a visual analog scale, 100 mm in length, marked “extremely bad” at 0 mm, and “excellent” at 100 mm. Subjective insomnia was evaluated by asking subjects to estimate the time spent awake during the previous night (Latshang et al., 2012, Nussbaumer-Ochsner et al., 2012c). Sleepiness was assessed by the Karolinska Sleepiness Scale which evaluates the current tendency to fall asleep on a scale ranging from 1 (very awake) to 9 (very tired) (Kaida et al., 2006). The mid-sleep time corrected for any sleep debt was determined as a measure of the individual chronotype by computing the midpoint between self-reported sleep onset and awakening on free days and work days, respectively, over the last few weeks (Roenneberg et al., 2004). Lung function assessment included spirometry, sniff nasal pressure and single breath carbon monoxide diffusing capacity (Society, 1995, Uldry and Fitting, 1995).

Outcomes and sample size estimation

Primary outcomes were the apnea/hypopnea index (AHI) and the PVT response speed (reciprocal value of the response time) (Basner and Dinges, 2011). Secondary outcomes were variables reflecting the breathing pattern, sleep structure, other measures of vigilance and cognitive performance. Minimally important differences in the AHI and in the PVT response speed were assumed as 10/h (SD 20/h) (Nussbaumer-Ochsner et al., 2012a) and 0.125 1/s (SD 0.25) (Basner and Dinges, 2011), respectively. To achieve a power of 80%, with a two-sided significance level of 0.05 and a drop-out rate of 5%, the required sample size was estimated to be 50.

Data analysis and statistics

Data distribution was evaluated by Shapiro Wilks statistics. As most outcome variables were not normally distributed all data are summarized as medians (quartiles). Occasional missing values were replaced by the corresponding median value of the group. The effects of altitude and time at any altitude were evaluated

by Friedman ANOVA followed by Wilcoxon matched pairs tests if P ANOVA was significant.

Generalized least square regression and ordered logistic regression analyses were employed to assess effects of altitude on the AHI, and measures of sleep, vigilance and psychomotor performance while controlling for various potential confounders. Outcomes were mathematically transformed to obtain a normal distribution or, if this was not feasible, they were divided into quintiles. Predictor variables for which univariable analysis indicated an association with a probability of $P<0.2$ were entered into a subsequent multivariable model.

Statistical significance was assumed at $P<0.05$ applying a Bonferroni correction as appropriate. Further details of the methods and of the statistical analysis are provided in the online supplement.

Results

Subjects

Of 190 screened subjects, 51 healthy men met the inclusion criteria and were randomized (see patient flow, figure 1).

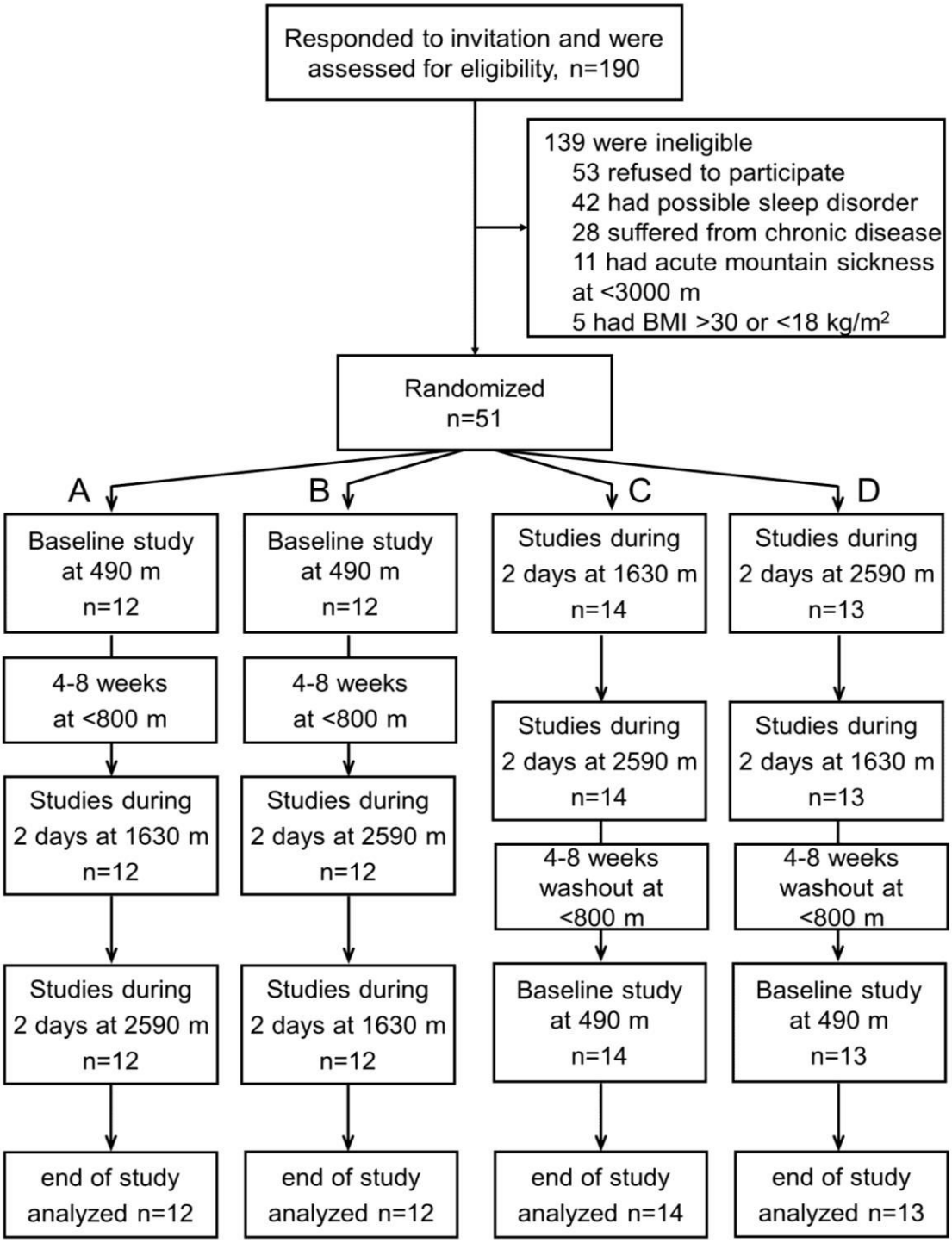

Figure 1: Patient flow according to the 4 different altitude exposure sequences A-D.

Their median age was 24 (quartiles 20 to 28) years, body mass index 23.0 (quartiles 21.0 to 24.8) kg/m^2, and Epworth sleepiness score 7 (quartiles 4 to 9, maximal value 10). Their self-reported median bed-time and get-up time on work days were 22:45 (22:00 to 23:00) and 07:00 (06:30 to 7:45) with an estimated

sleep duration of 8.0 (7.5 to 8.3) h; bed-time on free days was 01:00 (00:30 to 03:00), get-up time was 09:00 (09:00 to 10:00,) and estimated sleep duration was 9.0 (8.0 to 9.5) h. The mid-sleep time was 04:30 (03:58 to 05:11). Actigraphy confirmed a minimal duration of nocturnal rest time >7 h in all participants and a median rest time of 7.8 (quartiles 7.4-8.4) h in the week before the study.

Sleep studies

Results of sleep studies are summarised in table 1.

Table 1. Sleep studies

	490 m	1630 m		2590 m		P Friedman ANOVA
		1st night	2nd night	1st night	2nd night	
AHI, total, 1/h	4.6 (2.3;7.9)	7.0* (4.1;12.6)	5.4* (3.5;10.5)	13.1*†‡ (6.7;32.1)	8.0*†‡¶ (4.4;23.1)	<0.001
AHI obstructive, 1/h	1.3 (0.3;4.6)	1.8 (0.6;4.4)	2.7 (1.5;5.2)	1.8 (0.7;3.8)	1.6 (0.7;3.4)	0.010
AHI central, 1/h	2.0 (1.2;3.7)	4.6* (2.3;7.9)	2.8*† (1.7;5.1)	8.9*†‡ (5.0;25.8)	5.8*†‡¶ (2.8;13.1)	<0.001
ODI (>3%), 1/h	0.3 (0.0;1.1)	1.6* (0.5;3.8)	1.8* (0.5;3.8)	8.1*†‡ (3.3;30.9)	5.4*†‡¶ (2.5;14.8)	<0.001
Mean nocturnal SpO_2, %	96 (95;96)	94* (93;95)	94* (93;95)	90*†‡ (89;91)	91*†‡¶ (90;92)	<0.001
Time SpO_2 < 90%, %	0 (0;0)	0* (0;0)	0 (0;0)	36*†‡ (9;70)	16*†‡¶ (2;40)	<0.001
Mean inspiratory flow (Vt/Ti), L/s	0.16 (0.13;0.18)	0.17 (0.14;0.20)	0.16 (0.12;0.19)	0.18* (0.14;0.24)	0.17 (0.14;0.20)	0.046
Minute ventilation, L/min	3.95 (3.14;4.49)	4.23 (3.74;4.91)	3.95 (3.20;5.54)	4.06* (3.52;6.48)	4.47*† (3.81;5.18)	0.006
Tidal volume, L	0.24 (0.21;0.29)	0.27 (0.22;0.33)	0.27 (0.21;0.34)	0.28* (0.22;0.40)	0.29* (0.24;0.34)	0.015
Breath rate, 1/min	15 (14;16)	15 (14;17)	15 (14;17)	16‡ (14;18)	16*†‡(14;18)	<0.001
End-tidal PCO_2, mmHg	41(39;44)	38*(37;40)	39*(37;40)	37*†‡(34;38)	36*†‡(34;37)	<0.001
Heart rate, 1/min	56 (50;61)	56 (51;61)	56 (51;60)	60*†‡(55;65)	61*†‡(56;65)	<0.001
Total sleep time, min	399 (386;412)	400 (372;411)	408*† (400;414)	402‡ (384;410)	405 (388;413)	0.011
Sleep latency, min	13 (8;21)	12 (7;16)	10*† (7;12)	9* (7;13)	9* (7;12)	<0.001
Sleep efficiency, %	97 (95;100)	98 (91;99)	99 (98;100)	98 (94;99)	98 (96;99)	0.077
Wakefulness after sleep onset, min	11 (3;22)	12 (4;39)	7 (3;11)	11 (4;25)	9 (3;22)	0.096
NREM 1+2, %	56 (51;63)	55‡ (49;61)	50*†(42;53)	58‡(52;64)	55‡(51;62)	<0.001
NREM 3+4, %	24(20;27)	24(19;26)	24(20;29)	20*†‡(16;24)	21‡(18;25)	<0.001
REM, %	19 (15;24)	22 (18;26)	26*†(21;29)	20‡(18;25)	22*‡(19;26)	<0.001
Arousal index, 1/h	8.3 (6.1;9.6)	6.5 (5.3;9.3)	6.8(5.5;8.3)	7.7‡(6.1;9.7)	7.7 (6.1;10.9)	0.005

Medians (quartiles), n=51. AHI= apnea/hypopnea index; SpO_2,=oxygen saturation; ODI=oxygen desaturation index.* P<0.05 vs 490 m, † P<0.05 vs. 1630 m day 1, ‡ P<0.05 vs. 1630 m day 2. ¶ P<0.05 vs. 2590 m, day 1.

Compared to values recorded at 490 m, the nocturnal oxygen saturation was significantly reduced at 1630 m and at 2590 m in an altitude dependent way. While the median AHI was normal at 490 m its values were increased significantly already at 1630 m and even more at 2590 m. This was predominantly related to the emergence of central apnea/hypopnea. There was a considerable interindividual variability of the oxygen saturation and of the AHI with individual maximal AHI of 25.4/h at 490 m, of 39.5/h at 1630 m, and of 100.8/h at 2590 m (figure 2A).

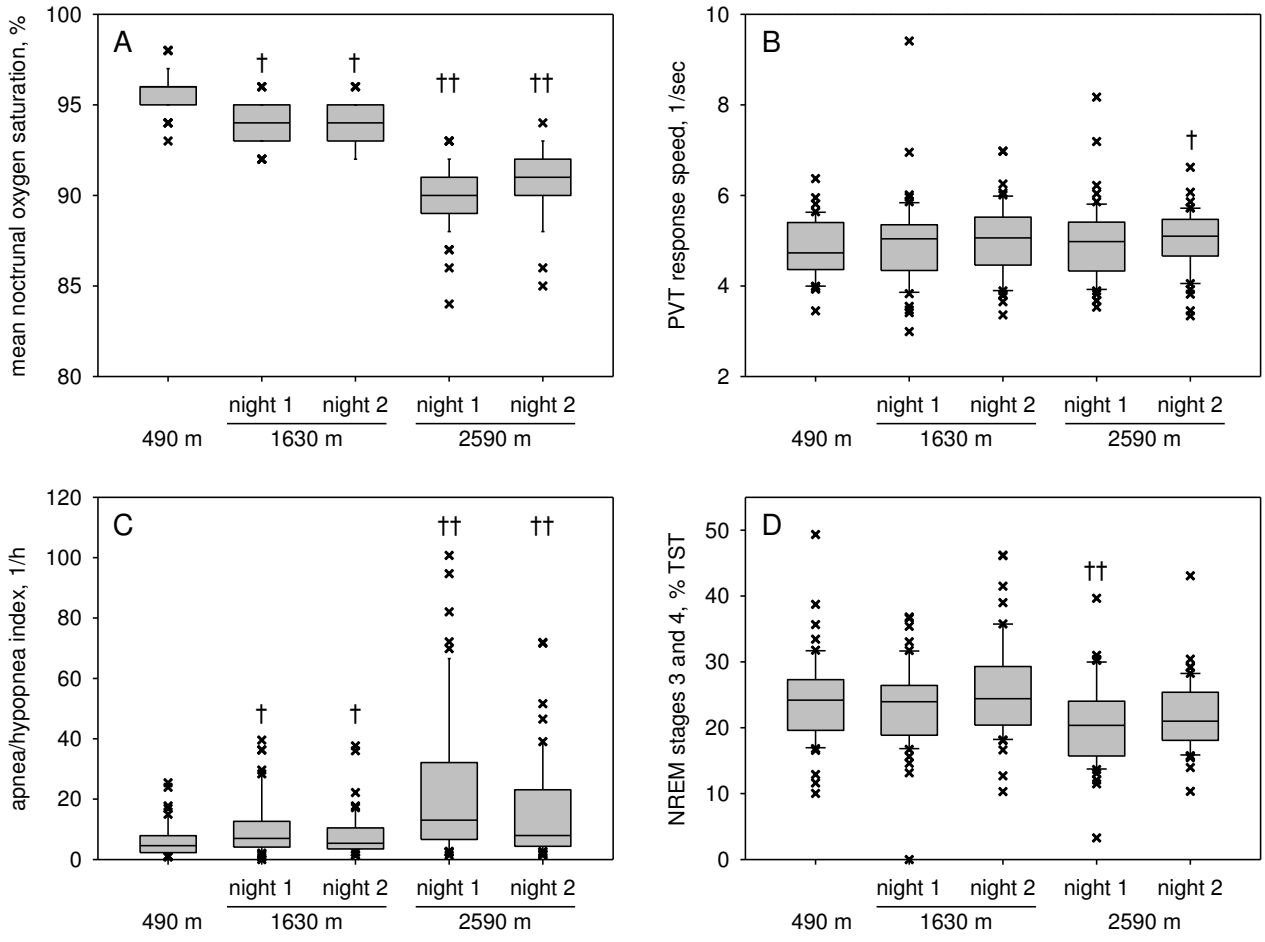

Figure 2: The Apnea/hypopnea index (A), psychomotor vigilance test response speed (B), mean nocturnal oxygen saturation (C), and slow wave sleep (D, NREM sleep stages 3 and 4 in % of total sleep time) at the different altitudes. Horizontal lines, boxes and whiskers represent the median, quartiles and the 10^{th} and 90^{th} percentiles, respectively; individual values beyond this range are displayed by an asterisk. † indicates $P<0.05$ vs. 490 m, †† indicates $P<0.05$ vs. 490 m and 1630 m, respectively.

Compared to the first night at 2590 m, the oxygen saturation was higher and the total and central AHI were lower in the second night at 2590 m suggesting some acclimatization. Mean inspiratory flow (tidal volume/inspiratory time), a measure of ventilatory drive (Tobin et al., 1988) increased at altitude due to an increase in both tidal volume and breath rate. Correspondingly, end tidal carbon dioxide tension ($PetCO_2$), the surrogate of the arterial PCO_2, decreased with increasing altitude.

Sleep stage specific analysis revealed an increase in the central AHI during non-rapid eye movement (NREM) sleep at 1630 m and 2590 m and during rapid eye movement (REM) sleep at 2590 m compared to 490 m (table E1, online supplement). Oxygen saturation was reduced to a similar degree in NREM and REM sleep. Mean inspiratory flow and minute ventilation were increased during REM sleep at 1630 m and 2590 m and this was associated with a reduction in $PetCO_2$.

To investigate the determinants of the AHI at 1630 m and 2590 m generalized least square regression analysis was performed. Apart from altitude, variables derived from polysomnography (mean nocturnal oxygen saturation, end-tidal PCO_2), the forced vital capacity (FVC), the number of days spent at altitude, altitude exposure sequence, and baseline characteristics (age, body mass index, AHI and nocturnal oxygen saturation at 490 m) were entered into the analysis. The results confirmed a positive association of the AHI with altitude, and a negative association of the AHI with the number of days at altitude (table 3).

Table 3. Generalized least square regression analysis of the effect of altitude exposure on the apnea/hypopnea index

Dependent variable Log10(AHI)	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coeffi-cient	95% CI	P
Altitude						
1630 m vs. 490 m	0.160	0.077 to 0.243	<0.001	0.200	0.039 to 0.362	0.015
2590 m vs. 490 m	0.396	0.290 to 0.502	<0.001	0.337	0.001 to 0.674	0.049
Mean nocturnal oxygen saturation, %	-0.064	-0.082 to -0.047	<0.001	-0.019	-0.074 to 0.036	0.493
PetCO2, mmHg	-0.031	-0.043 to -0.020	<0.001	-0.010	-0.022 to 0.003	0.123
FVC, % predicted	-0.001	-0.008 to 0.006	0.719			
Number of days at altitude						
2^{nd} vs. 1^{st} day	-0.167	-0.252 to -0.081	<0.001	-0.163	-0.258 to -0.068	0.001
3^{rd} vs. 1^{st} day	-0.092	-0.224 to 0.039	0.169	-0.099	-0.214 to 0.015	0.089
4^{th} vs. 1^{st} day	-0.154	-0.278 to -0.030	0.015	-0.151	-0.268 to -0.033	0.012
Altitude exposure sequence	-0.026	-0.111 to 0.059	0.542			
Age	0.016	0.010 to 0.023	<0.001	0.005	-0.005 to 0.015	0.344
Body mass index, kg/m^2	0.026	-0.013 to 0.066	0.184			
AHI at 490 m	0.041	0.029 to 0.052	<0.001	0.037	0.025 to 0.048	<0.001
Mean nocturnal oxygen saturation at 490 m	-0.076	-0.147 to -0.004	0.038	-0.016	-0.089 to 0.056	0.663

n=255 observations (51 participants) at 490, 1630 and 2590 m. Results are presented for the logarithmically (log 10) transformed apnea/hypopnea index (AHI) as dependent variable. Independent variables with P<0.2 in the univariable analysis were included into the multivariable analysis. The multivariable model was adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude was 1-4; altitude exposure sequences were A-D, see figure 1. CI=confidence interval. PetCO2=mean nocturnal transcutaneous PCO_2; FVC=forced vital capacity.

Table 4. Apnea/hypopnea indices predicted for men of different age by a regression model

Predicted AHI at specified age, 1/h	490 m	1630 m		2590 m		P regression
		1st night	2nd night	1st night	2nd night	
Age 20 y (10th percentile)	5.2 (3.3 to 8.0)	8.2 (6.1 to 11.0)	5.6 (4.3 to 7.4)	8.9 (6.0 to 13.3)	7.9 (5.5 to 11.4)	<0.001
Age 24 y (50th percentile)	5.4 (3.6 to 8.2)	8.6 (6.6 to 11.2)	5.9 (4.6 to 7.6)	9.3 (6.4 to 13.6)	8.3 (5.9 to 11.6)	<0.001
Age 38 y (90th percentile)	6.3 (3.9 to 10.3)	10.0 (6.8 to 14.8)	6.9 (4.6 to 10.5)	10.9 (6.9 to 17.3)	9.7 (6.3 to 14.9)	<0.001

The predicted apnea/hypopnea indices (AHI) with their 95% confidence intervals were computed based on the regression model presented in table 3 for men of 3 different ages ascending from 490m to 1630m and 2590m.

In addition, the AHI at 490 m was a significant positive predictor of the AHI at altitude while the body mass index was not . To illustrate the increase in AHI with increasing altitude and age, the regression model (table 3) was used to predict the AHI at the 3 altitudes for 3 distinct ages (the 10th, the 50th, and the 90th percentile of the age distribution of all subjects, i.e., 20 y, 24 y and 28 y). An exploratory analysis restricted to a more homogeneous group of 43 younger subjects (20 to 29 y of age) did not reveal results that differed principally from those obtained in all 51 subjects in terms of the major outcomes, mean nocturnal oxygen saturation, apnea/hypopnea index (data not shown).

Sleep continuity was high at all altitudes as reflected in a high sleep efficiency, a short time in wakefulness after sleep onset and a low arousal index (table 1). Compared to values at 490 m there were slight but statistically significant changes in sleep structure consisting in a reduction in slow wave sleep (NREM stages 3 and 4) at 2590 m (figure 2D), and an increase in REM sleep in the second night at 1630 m and 2590 m, respectively (table 1). The altitude related sleep disturbances were further corroborated by significant independent effects of altitude on slow wave sleep and on the arousal index in multiple regression analyses, respectively (tables E2 and E3, online). Consistent with the minimal and statistically non-significant changes in wakefulness after sleep onset and in sleep efficiency, regression analyses did not suggest any independent effect of altitude on these outcomes (tables E4 and E5, online supplement). While age was a significant independent predictor of some of the sleep variables the body mass index and the mid-sleep time were not (tables E2-E5).

Daytime evaluation

Daytime evaluations are summarized in table 2.

Table 2. Daytime evaluation

	490 m	1630 m		2590 m		P Friedman ANOVA
		1st day	2nd day	1st day	2nd day	
Vigilance and cognitive performance						
PVT response speed (1/RT), 1/s	4.7 (4.4;5.4)	5.0 (4.3;5.3)	5.1 (4.5;5.5)	5.0 4.3;5.4)	5.1* (4.7;.5)	0.015
PVT number of lapses	1 (0;4)	2 (0;3)	1 (0;3)	1 (0;3)	1 (0;3)	0.069
DASS, reaction time, s	1.9 (1.5;2.3)	2.1 (1.6;2.6)	2.1 (1.6;2.6)	1.9 (1.5;2.4)	1.8 (1.4;2.6)	0.389
DASS, tracking error, arbitrary units	0.30 (0.21;0.39)	0.27 (0.22;0.40)	0.29* (0.20;0.35)	0.26 (0.20;0.35)	0.24*†‡¶ (0.19;0.30)	<0.001
1, 2, 3 back mean reaction time of correct answers, ms	637 (554;793)	674 (594;828)	635† (554;713)	674 (579;769)	628 (550;734)	0.019
1, 2, 3 back correct answers, %	92 (89;95)	93* (90;96)	93* (90;95)	93 (90;95)	93 (91;95)	0.031
Trail making test, s	52.4 (43.8;58.9)	48.1 (42.5;58.5)	46.5*† (40.7;55.5)	51.2 (43.5;58.4)	48.0*¶ (40.8;55.5)	0.003
Questionnaire evaluation						
Estimated night-time spent awake, min	20 (10;40)	20 (10;30)	10*† (5;20)	20‡ (10;50)	15‡ (5;30)	<0.001
Sleep quality, Visual analog score	6.2 (4.0;7.5)	6.0 (4.3;7.5)	7.1*† (6.0;8.2)	5.5‡ (3.8;7.0)	6.3* (5.1;7.9)	0.006
Karolinska sleepiness score ¥	3 (2;4)	3 (3;5)	3 (3;5)	3 (3;5)	3 (3;4)	0.192
Acute mountain sickness score (AMSc)	0.00 (0.00;0.10)	0.00 (0.00;0.09)	0.00 (0.00;0.00)	0.00 (0.00;0.00)	0.00 (0.00;0.00)	0.194

Medians (quartiles), n=51. PVT=psychomotor vigilance test; DASS=divided attention steering simulator;
* P<0.05 vs. 490 m, † P<0.05 vs. 1630 m day 1, ‡ P<0.05 vs. 1630 m day 2. ¥ Subjective sleepiness rated from 1 (very awake) to 9 (very tired).

There were no consistent changes in the outcomes of the PVT (figure 2B), the divided attention steering simulator test, the number back test and the trail making test at 1630 m and 2590 m compared to baseline values at 490 m. To evaluate whether any potential effect of altitude on tests of vigilance and psychomotor performance was masked by confounding effects of time (i.e., including learning or acclimatization effects), the sequence of altitude exposure or disturbances of breathing and sleep in the previous night multivariable regression analyses were performed. These analyses did not reveal any independent effect of altitude exposure on outcomes when controlled for covariables (tables E6-E9, online supplement).

According to the questionnaires subjects did not feel that they had spent more time awake during nights at 1630 m and 2590 m compared to 490 m (table 2). They rated their sleep quality as slightly better in the second night at 1630 m and 2590 m compared to 490 m. Subjects were not particularly sleepy as assessed by the Karolinska sleepiness scale and their rating remained unchanged during the study. None of the subjects suffered from symptoms of acute mountain sickness (table 2, AMSc questionnaire) such as lost appetite, headaches, faintness, for example, as evidenced by a median score of the corresponding items of 0 (quartiles 0, 0) with item scores ranging from 0=”not at all” to 5=”extreme”.

Spirometry revealed a decrease in forced vital capacity by 4 % predicted and an increase in the ratio of the forced expiratory volume in one second to vital capacity (FEV1/FVC) 2590 m while the nasal sniff pressure and diffusing capacity were unchanged compared to 490 m (online supplement Table E10).

Discussion

Our randomized crossover trial in a relatively large cohort of healthy young men living near sea level reveals a considerable and individually highly variable amount of sleep related periodic breathing associated with mild hypoxemia during acute exposure to altitudes of 1630 m and 2590 m for a total of 4 days. Mild alterations in sleep structure and in subjective sleep quality were also noted. Performances in a battery of cognitive and psychomotor vigilance tests were not consistently changed. These novel results are important since they are pertinent to a vast number of persons travelling to moderate altitude worldwide.

There are few published reports on sleep, breathing and daytime performance at moderate altitude, and studies are heterogeneous including only small groups of subjects. Mizuno and co-workers (Mizuno et al., 1993) did not observe significant changes in the AHI and in sleep structure in 5 normal men undergoing polysomnography in a hypobaric chamber at sea level and at simulated altitudes of 1500 m and 3000 m, possibly related to an inadequate sample size. Muhm and co-workers (Muhm et al., 2009) performed a randomized, double-blind cross-over study in 20 healthy men undergoing simulated air travel in a hypobaric chamber for 14 h including an 8 h night rest. At the altitude equivalent of 2438 m, nocturnal oxygen saturation fell to 86 % and the AHI increased to 13/h, similar to values at 2590 m in the current study. No changes in sleep structure and in neurobehavioural tests performed in the following morning at the same altitude equivalent were noted (Muhm et al., 2009). Field studies and simulations in unacclimatized subjects sleeping at greater altitudes (>4000 m) (Beaumont et al., 2004, de Aquino Lemos et al., 2012, Eichenberger et al., 1996, Nussbaumer-Ochsner et al., 2012c) or in trekkers ascending gradually up to 5050 m (Johnson et al., 2010) suggest alterations in sleep structure with a reduction in sleep efficiency, deep sleep, and an increase in arousals in association with a low oxygen saturation and periodic breathing. Evaluation of cognitive and psychomotor performance at high altitude (>3500 m) has revealed conflicting results with some studies suggesting an impairment in cognitive performance, attention and mood (de Aquino Lemos et al., 2012, Mackintosh et al., 1988) while other showing no consistent changes (Beaumont et al., 2007, Luks et al., 1998, Regard et al., 1991). This may relate to the lack of randomization of the order of altitude exposure and small sample size in certain studies.

Our study extends the cited observations in several ways. The strength of the current randomized trial includes its robust design and blinded data analysis, the realistic field setting at two different moderate altitudes that are relevant for a very large number of travellers to destinations worldwide and the statistical power due to the inclusion of a large number of participants. Our data demonstrate that the mild hypoxemia at 1630 m and 2590 m was sufficient to induce periodic breathing with very high numbers of central apneas/hypopneas in certain individuals (i.e., an AHI up to 39.5/h at 1630 m, and up to 100.8/h at 2590 m, figure 2A). The elevations in the AHI at 1630 m and 2590 m were associated with an altitude

dependent decrease in oxygen saturation, an increase in mean inspiratory flow (the measure of ventilatory drive) and minute ventilation, and a decrease in $PetCO_2$. This is consistent with respiratory control theory suggesting that breathing is destabilized by hypoxic stimulation of ventilation, a high sensitivity to CO_2 and hypoxia along with a reduced CO_2 reserve (Dempsey et al., 2010). Multivariable regression analysis revealed that the AHI at 490 m was a significant predictor of the AHI at 1630 m and 2590 m (table 3) suggesting that the propensity for unstable control of breathing was present in certain subjects already at low altitude. This would be in line with the major increase in the AHI observed in the same altitude setting in patients with pre-existing obstructive sleep apnea syndrome (Nussbaumer-Ochsner et al., 2012a, Nussbaumer-Ochsner et al., 2010). The increase in oxygen saturation in the second day at 2590 m associated with a decrease in the AHI as well as the negative correlation of the AHI with the number days spent at 1630 m and 2590 m (table 3) indicates that acclimatization took place. While AHI decreased over the course of the 4 days at moderate altitude, observations at much higher altitudes (3750 m to 6850 m) revealed a persistent increase in periodic breathing over the course of 2 weeks (Bloch et al., 2010).

The trends of changes in sleep structure after ascent to 2590 m were similar in terms of reduction in slow wave sleep, but less pronounced compared to the changes we observed recently at 4559 m (Nussbaumer-Ochsner et al., 2012c). In contrast to the findings at higher altitude the sleep efficiency and measures of sleep continuity (arousal index and wakefulness after sleep onset) were not significantly affected at 1630 m and 2590 m (table 1).

The modest sleep disturbances, mild hypoxemia and the nocturnal periodic breathing did not result in measurable impairments of vigilance, cognitive and psychomotor performance at 1630 m and 2590 m although an extensive battery of tests evaluating different aspects of reaction, divided attention, cognitive performance and memory was employed. Our study was powered to detect a reduction in PVT reaction speed of less than 0.125/s, a change reported to occur after restricting sleep to 4 h for one night (Basner and Dinges, 2011). These results are reassuring as larger effects of acute exposure to moderate altitude on the PVT reaction speed did not occur in our subjects as a group. However, we cannot exclude that individual susceptible subjects might experience subtle alterations in neurophysiological and psychomotor function not detected by our tests although

still relevant in the daily activities of mountain travelers, drivers, air crew and in other settings. Multivariable regression analyses indicated an improvement in tests of vigilance, cognitive and psychomotor performance with each additional day at altitude. This might have been related to learning, acclimatization, or both (tables E6-9). However, no correlation of any of these outcomes with the AHI or nocturnal oxygen saturation was found. We therefore do not have evidence that altitude induced breathing disturbances caused cognitive impairments. In addition to elucidating the effects of altitude, our study provides a valuable set of normative data for the battery of cognitive and psychomotor performance tests that were applied to a large cohort of healthy subjects at low altitude.

Questionnaire evaluations revealed no symptoms of acute mountain sickness including no aspects of fatigue or excessive sleepiness at 1630 m and 2590 m compared to 490 m, and neither the subjective sleep quality nor the estimated time spent awake at night were altered in participants of the current study during their altitude sojourn (table 2). In contrast, patients with obstructive sleep apnea perceived having spent more time awake at 2590 m than at 490 m, possibly related to their severe hypoxemia, breathing and sleep disturbances (Nussbaumer-Ochsner et al., 2010). Our study was performed in healthy young men during 4 days at moderate altitude. Whether similar or more pronounced changes occur in older persons, in women or in patients with a pre-existing respiratory or cardiovascular condition. requires further study. Although our findings do not suggest an interaction of altitude effects with the chronotype we cannot exclude that altitude exposure is associated with alterations of the circadian rhythm (Coste et al., 2009, Urner et al., 2009).

In conclusion, the current randomized trial performed in a large cohort of healthy men provides robust evidence that nocturnal breathing and sleep are disturbed in the first 4 nights after ascent to 1630 m and 2590 m. The amount of periodic breathing is highly variable and predicted in part by the AHI at low altitude. The finding that measures of vigilance, cognitive and psychomotor performance were not altered to a measurable degree is particularly relevant for the large number of persons travelling to moderate altitude worldwide as well as for air crew. Nevertheless, susceptible persons, the elderly or patients with obstructive sleep apnea syndrome and other pre-existing breathing disorder might still experience adverse effects of moderate altitude.

Online Data Supplement

Methods

Monitoring of ventilation during sleep

Apneas/hypopneas were detected by nasal prong pressure recordings and by calibrated respiratory inductive plethysmography (RIP) (Thurnheer et al., 2001). RIP was calibrated in the evening as previously described (Bloch et al., 2010, Bloch et al., 1997). After a qualitative diagnostic calibration a fixed volume calibration was performed by letting subjects rebreathe into a bag of 800 ml volume (Sackner et al., 1989). Accuracy of volume calibration was verified in the morning after sleep studies by rebreathing into the 800 ml bag. If tidal volume deviated by >20% from this volume the variables depending on this calibration were omitted from analysis. Additional respiratory monitoring included diaphragmatic surface EMD (Maarsingh et al., 2000), capnography of expired air (Nussbaumer-Ochsner et al., 2012a) and pulse oximetry.

Breath by breath measurement of breathing pattern characteristics (breath rate, tidal volume, minute ventilation and mean inspiratory flow) and of end-tidal carbon dioxide tension were assessed with dedicated software (EDP V4.2, Non-invasive Monitoring Systems, Miami Beach, FL) as described previously (Bloch et al., 1997). Apneas/hypopneas were defined as a reduction of the inductive plethysmographic sum signal or the nasal pressure swings to <50% of the preceding 2 min baseline during ≥10 s. Transient reductions in breathing amplitude to <50% baseline for 5-10 s were also scored as apneas/hypopneas if they occurred as part of a periodic breathing pattern with hyperventilation alternating with central apneas/hypopneas for at least three consecutive cycles (Bloch et al., 2010). Obstructive apneas/hypopneas were identified by rib cage and abdominal asynchrony and persistent or increasing diaphragmatic EMG activity (Nussbaumer-Ochsner et al., 2012a). Central apneas/hypopneas were identified by absent rib cage-abdominal asynchrony, no signs of inspiratory flow limitations (no flattening of nasal pressure contour) and reduced or absent diaphragmatic EMG activity. Mixed apneas/hypopneas that showed some characteristics suggesting upper airway obstruction were classified as obstructive events. The apnea/hypopnea index and

the oxygen desaturation index (ODI, >3% dips) were computed as the number of events per hour of sleep.

Data analysis and statistics

The normality of distribution of outcomes was evaluated by the Shapiro-Wilks statistic (Kirkwood and Sterne, 2003). This revealed that the majority of outcome variables were not normally distributed. Therefore, all data are summarized by medians and quartiles. Data were grouped according to altitude, and overall effects were evaluated by Friedman analysis of variance (ANOVA). If ANOVA revealed a significant overall effect planned post hoc analyses were performed using Wilcoxon matched pairs tests.

Regression analyses were employed to separately assess the effect of altitude (1630 m and 2590 m vs. 490 m) on major outcomes while controlling for several potential confounders. For certain variables a normal distribution was achieved by the following transformation: logarithm (log 10) or 1/square root. The transformed variables revealing a normal distribution were entered into univariable and multivariable random effects generalized least square regression analyses. If an outcome variable was not normally distributed and mathematical transformation to a normal distribution could not be achieved quintiles of that variable were entered as the dependent variable into univariable and multivariable ordered logistic regression analyses using robust standard errors. All predictor variables for which univariable analysis indicated an association with a probability of $P<0.2$ were entered into a subsequent multivariable model.

In a first regression analysis potential predictors of the AHI were evaluated. The dependent variable was the AHI (log 10 transformed); the independent variables (predictors) were altitude (490 m=1, 1630 m=2, 2590 m=3), mean nocturnal oxygen saturation, end-tidal PCO_2 (the surrogate of the arterial PCO_2), forced vital capacity (FVC), number of days at altitude (1 to 4), altitude exposure sequence (A to D, see figure 1), age, AHI at 490 m, mean nocturnal oxygen saturation at 490 m.

Additional regression analyses were performed to evaluate the predictors of variables reflecting sleep structure (slow wave sleep; arousal index; sleep efficiency; wakefulness after sleep onset), and of performance in tests of vigilance and psychomotor performance. Dependent variables were: PVT response speed

(quintiles of the reciprocal value of reaction time), divided attention steering simulator tracking error (log 10 transformed SD of mean deviation from the center line); response time in the 1-, 2-, 3- back test (log 10 transformed); time to complete the trail making test (transformed by computing 1/square root). The independent variables in these analyses were: altitude (490 m=1, 1630 m=2, 2590 m=3), mean nocturnal oxygen saturation, slow wave sleep duration (time in NREM stages 3 and 4), the AHI, the number of days at altitude (1 to 4), altitude exposure sequence (A to D), age.

A probability of $P<0.05$ applying a Bonferroni correction was considered as statistically significant. Analyses were performed with Statistica V8.0, StatSoft, Tulsa, USA, and Stata 11.1, StataCorp, College Station, USA.

Table E1. Sleep stage specific oxygen saturation and ventilation

	490 m	1630 m		2590 m		P Friedman ANOVA
		1st night	2nd night	1st night	2nd night	
Obstructive AHI NREM, 1/h	0.5§(0.0;2.8)	0.6§ (0.0;1.8)	1.0§ (0.4;2.2)	0.9§ (0.0;2.2)	0.5§ (0.0;2.2)	0.510
Obstructive AHI REM, 1/h	4.1 (0.0;9.8)	5.8 (1.0;11.0)	5.3 (2.9;12.5)	4.4 (1.8;11.0)	4.8 (2.1;7.6)	0.154
Central AHI NREM, 1/h	2.1 (0.9;3.6)	5.1* (2.0;8.5)	2.9*† (1.6;5.5)	9.3*†‡§ (4.7;31.6)	5.4*†‡¶§ (2.9;13.3)	<0.001
Central AHI REM, 1/h	1.8 (0.8;4.5)	3.5 (1.4;6.9)	2.3 (1.2;4.4)	6.9*‡ (3.6;13.2)	3.6*‡ (1.7;9.1)	<0.001
SpO_2 during wakefulness, %	96 (96;97)	94* (94;95)	95* (94;95)	91*†‡ (90;92)	92*†‡¶ (91;93)	<0.001
SpO_2 during NREM, %	96§ (95;96)	94*§ (93;94)	94*§ (93;94)	90*†‡ (89;91)	91*†‡¶ (90;92)	<0.001
SpO_2 during REM, %	96 (96;97)	94* (93;95)	94* (94;95)	90*†‡ (88;91)	91*†‡¶ (90;92)	<0.001
Vt/Ti awake	0.22 (0.18;0.26)	0.24 (0.21;0.33)	0.24 (0.19;0.31)	0.22 (0.18;0.37)	0.25 (0.19;0.35)	0.297

Vt/Ti NREM	0.15 (0.12;0.19)	0.16 (0.14;0.20)	0.16 (0.12;0.20)	0.18* (0.14;0.25)	0.18* (0.14;0.21)	0.012
Vt/Ti REM	0.16 (0.13;0.19)	0.18 (0.14;0.22)	0.17 (0.13;0.21)	0.16 (0.13;0.24)	0.18 (0.14;0.22)	0.343
V`E awake, L/min	6.03 (4.50;6.57)	5.87 (5.41;7.01)	5.98 (4.92;7.67)	5.58 (4.55;8.13)	5.81 (4.79;7.77)	0.902
V`E NREM, L/min	3.88§ (3.02;4.75)	4.11§ (3.64;4.65)	3.71§ (3.07;5.27)	3.96*§ (3.46;6.32)	4.32*†§ (3.75;5.06)	<0.002
V`E REM, L/min	4.52 (3.64;5.49)	4.91 (4.22;6.00)	4.87 (3.64;6.16)	4.62 (3.84;6.68)	4.84 (3.99;5.66)	0.240
Vt awake, L	0.31 (0.28;0.44)	0.38 (0.34;0.44)	0.35 (0.25;0.43)	0.35 (0.29;0.48)	0.35 (0.28;0.46)	0.383

Table E1, continued

	490 m	1630 m		2590 m		
		1st night	2nd night	1st night	2nd night	P
Vt NREM, L	0.24§ (0.20;0.29)	0.26§ (0.22;0.32)	0.26§ (0.19;0.33)	0.28* (0.22;0.42)	0.29* (0.23;0.34)	0.012
Vt REM, L	0.27 (0.25;0.35)	0.30 (0.24;0.37)	0.30 (0.22;0.40)	0.30 (0.24;0.39)	0.29 (0.24;0.35)	0.797
Breath rate awake, 1/min.	16 (15;19)	16 (15;18)	17 (15;19)	16 (15;18)	17 (15;18)	0.395
Breath rate NREM, 1/min.	15§ (14;17)	15§ (14;17)	15§ (14;17)	15‡§ (14;17)	16*†‡§ (14;17)	<0.001
Breath rate REM, 1/min.	16 (14;17)	16 (15;19)	16 (14;18)	16*‡ (15;18)	17*‡ (15;18)	<0.001
$PetCO_2$, awake, mmHg	40 (37;42)	37* (35;39)	37* (35;39)	36* (34;38)	35*†‡ (34;37)	<0.001
$PetCO_2$, NREM, mmHg	41§ (39;44)	39*§ (37;41)	39*§ (37;40)	37*†‡§ (34;39)	36*†‡§ (35;37)	<0.001
$PetCO_2$, REM, mmHg	41 (39;43)	38* (35;40)	38* (37;40)	37*‡ (33;38)	36*†‡ (34;37)	<0.001
Heart rate, 1/min	56 (50;61)	56 (51;61)	56 (51;60)	60*†‡ (55;65)	61*†‡ (56;65)	<0.001
Premature beats, entire night, 1/h	2.7 (0.3;10.2)	3.0 (0.7;11.7)	3.3 (1.1;11.8)	1.7 (0.6;8.4)	2.0 (0.4;14.3)	0.101

Data are medians (quartiles), n=51. SpO_2= oxygen saturation. $PetCO_2$= end tidal carbon dioxide tension, AHI= apnea/hypopnea index. Vt=tidal volume, V`E=minute ventilation.

* P<0.05 vs. 490 m; † P<0.05 vs. 1630 m day 1; ‡ P<0.05 vs. 1630 m day 2; ¶ P<0.05 vs 2590 m day 1; § P<0.05 vs. REM.

Table E2. Logistic regression analysis of the effect of altitude exposure on the slow wave sleep

Dependent variable quintiles of slow wave sleep	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coeffi-cient	95% CI	P
Altitude						
1630 m vs. 490 m	0.079	-0.338 to 0.496	0.711	-0.978	-1.980 to 0.024	0.056
2590 m vs. 490 m	-0.798	-1.328 to -0.268	0.003	-2.644	-4.693 to -0.594	0.011
Mean nocturnal oxygen saturation, %	0.134	0.049 to 0.220	0.002	-0.249	-0.575 to 0.076	0.133
AHI, 1/h	-0.041	-0.072 to -0.010	0.009	-0.026	-0.053 to -0.002	0.067
Number of days at altitude						
2^{nd} vs. 1^{st} day	0.945	0.442 to 1.447	<0.001	1.060	0.476 to 1.644	<0.001
3^{rd} vs. 1^{st} day	0.389	-0.110 to 0.889	0.126	0.578	0.018 to 1.138	0.043
4^{th} vs. 1^{st} day	0.737	0.206 to 1.267	0.006	0.916	0.292 to 1.540	0.004
Altitude exposure sequence	0.067	-0.292 to 0.426	0.715			
Age	-0.073	-0.119 to -0.027	0.002	-0.076	-0.129 to -0.024	0.004
Body mass index, $kg*m^{-2}$	-0.066	-0.207 to 0.075	0.358			
Mid-sleep time, h	0.223	-0.174 to 0.620	0.271			

n=255 observations at 490, 1630 and 2590 m (51 participants). Results are presented for quintiles of slow wave sleep as dependent variable. Independent variables with P<0.2 in the univariable analysis were included into the multivariable analysis. The multivariable model was adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude was 1-4; altitude exposure sequences were A-D, see figure 1. CI=confidence interval; AHI=apnea/hypopnea index.

Table E3. Logistic regression analysis of the effect of altitude exposure on the arousal index

Dependent variable quintiles of arousal index, 1/h	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coeffi-cient	95% CI	P
Altitude						
1630 m vs. 490 m	-0.676	-1.141 to -0.212	0.004	-0.835	-1.667 to -0.003	0.049
2590 m vs. 490 m	-0.054	-0.463 to 0.355	0.796	-0.815	-2.564 to 0.935	0.361
Mean nocturnal oxygen saturation, %	-0.085	-0.164 to -0.007	0.034	-0.068	-0.331 to 0.196	0.614
AHI, 1/h	0.044	0.016 to -0.072	0.002	0.040	0.008 to 0.073	0.015
Number of days at altitude						
2^{nd} vs. 1^{st} day	-0.943	-0.599 to 0.411	0.714	0.222	-0.354 to 0.798	0.450
3^{rd} vs. 1^{st} day	-0.631	-1.208 to -0.054	0.032	-0.493	-1.08 to 0.093	0.099
4^{th} vs. 1^{st} day	-0.346	-0.976 to 0.283	0.281	-0.074	-0.763 to 0.616	0.834
Altitude exposure sequence	-0.031	-0.343 to 0.281	0.846			
Age	0.045	0.014 to 0.076	0.004	0.022	-0.019 to 0.063	0.291
Body mass index, $kg*m^{-2}$	0.060	-0.087 to 0.207	0.425			
Mid-sleep time,h	0.005	-0.360 to 0.369	0.980			

n=255 observations at 490, 1630 and 2590 m (51 participants). Results are presented for quintiles of arousal index per hour as dependent variable. Independent variables with P<0.2 in the univariable analysis were included into the multivariable analysis. The multivariable model was adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude was 1-4; altitude exposure sequences were A-D, see figure 1. CI=confidence interval; AHI=apnea/hypopnea index.

Table E4. Logistic regression analysis of the effect of altitude exposure on the wake after sleep onset

Dependent variable quintiles of wakefullness after sleep onset	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coeffi-cient	95% CI	P
Altitude						
1630 m vs. 490 m	-0.172	-0.686 to 0.343	0.513	0.619	-0.236 to 1.474	0.156
2590 m vs. 490 m	0.123	-0.320 to 0.566	0.585	0.878	-0.677 to 2.432	0.268
Mean nocturnal oxygen saturation, %	-0.084	-0.168 to 0.001	0.052	-0.025	-0.266 to 0.215	0.836
AHI, 1/h	0.019	0.001 to 0.037	0.038	-0.002	-0.017 to 0.014	0.846
Number of days at altitude						
2^{nd} vs. 1^{st} day	-0.966	-1.512 to -0.420	0.001	-1.079	-1.758 to -0.400	0.002
3^{rd} vs. 1^{st} day	-1.050	-1.668 to -0.432	0.001	-1.174	-1.885 to -0.463	0.001
4^{th} vs. 1^{st} day	-1.108	-1.644 to -0.573	<0.001	-1.182	-1.864 to -0.500	0.001
Altitude exposure sequence	-0.029	-0.377 to 0.320	0.872			
Age	0.082	0.042 to 0.121	<0.001	0.078	0.035 to 0.121	<0.001
Body mass index, $kg*m^{-2}$	0.143	0.031 to 0.255	0.012	0.066	-0.053 to 0.184	0.276
Mid-sleep time	-0.164	-0.549 to 0.221	0.404			

n=255 observations at 490, 1630 and 2590 m (51 participants). Results are presented for quintiles of wake after sleep onset as dependent variable. Independent variables with P<0.2 in the univariable analysis and altitude, the parameter of the main hypothesis were included into the multivariable analysis. The multivariable model was adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude was 1-4; altitude exposure sequences were A-D, see figure 1. CI=confidence interval; AHI=apnea/hypopnea index.

Table E5. Logistic regression analysis of the effect of altitude exposure on the sleep efficiency

Dependent variable quintiles of sleep efficiency	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coeffi-cient	95% CI	P
Altitude						
1630 m vs. 490 m	0.199	-0.314 to 0.712	0.447	-0.610	-1.512 to 0.293	0.186
2590 m vs. 490 m	-0.132	-0.624 to 0.360	0.599	-0.872	-2.414 to 0.669	0.267
Mean nocturnal oxygen saturation, %	0.091	-0.001 to 0.184	0.052	0.023	-0.239 to 0.285	0.864
AHI, 1/h	-0.021	-0.037 to -0.005	0.009	-0.002	-0.017 to 0.013	0.791
Number of days at altitude						
2^{nd} vs. 1^{st} day	1.161	0.539 to 1.783	<0.001	1.208	0.482 to 1.934	0.001
3^{rd} vs. 1^{st} day	1.017	0.377 to 1.656	0.002	1.106	0.373 to 1.839	0.003
4^{th} vs. 1^{st} day	1.234	0.605 to 1.863	<0.001	1.280	0.538 to 2.022	0.001
Altitude exposure sequence	0.001	-0.324 to 0.326	0.996			
Age	-0.073	-0.104 to -0.043	<0.001	-0.068	-0.101 to -0.036	<0.001
Body mass index, $kg*m^{-2}$	-0.135	-0.235 to -0.034	0.009	-0.066	-0.171 to 0.040	0.225
Mid-sleep time, h	0.174	-0.165 to 0.513	0.315			

n=255 observations at 490, 1630 and 2590 m (51 participants). Results are presented for quintiles of wake after sleep onset as dependent variable. Independent variables with P<0.2 in the univariable analysis and altitude, the parameter of the main hypothesis were included into the multivariable analysis. The multivariable model was adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude was 1-4; altitude exposure sequences were A-D, see figure 1. CI=confidence interval; AHI=apnea/hypopnea index.

Table E6. Logistic regression analysis of the effect of altitude exposure on psychomotor vigilance test (PVT)

Dependent variable quintiles of PVT response speed (1/reaction time)	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coeffi-cient	95% CI	P
Altitude						
1630 m vs. 490 m	0.330	-0.130 to 0.790	0.159	-0.100	-0.607 to 0.407	0.699
2590 m vs. 490 m	0.317	-0.061 to 0.695	0.100	-0.066	-0.539 to 0.407	0.784
Mean nocturnal oxygen saturation, %	0.001	-0.080 to 0.082	0.979			
Slow wave sleep, min	0.012	0.000 to 0.024	0.049	0.009	-0.003 to 0.021	0.151
AHI, 1/h	-0.000	-0.021 to 0.020	0.981			
Number of days at altitude						
2^{nd} vs. 1^{st} day	0.449	0.149 to 0.748	0.003	0.332	-0.063 to 0.728	0.099
3^{rd} vs. 1^{st} day	0.731	0.332 to 1.130	<0.001	0.725	0.302 to 1.148	0.001
4^{th} vs. 1^{st} day	0.867	0.529 to 1.204	<0.001	0.782	0.404 to 1.160	<0.001
Altitude exposure sequence	0.157	-0.232 to 0.546	0.429			
Age	-0.035	-0.082 to 0.131	0.155	-0.026	-0.078 to 0.025	0.317

n=255 observations at 490, 1630 and 2590 m (51 participants). Results are presented for quintiles of the PVT response speed as dependent variable. Independent variables with P<0.2 in the univariable analysis are included into the multivariable analysis. The multivariable model is adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude was 1-4; altitude exposure sequences were A-D, see figure 1. CI=confidence interval.

Table E7. Generalized least square regression analysis of the effect of altitude exposure on driving simulator performance

Dependent variable Log10(tracking error)	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coeffi-cient	95% CI	P
Altitude						
1630 m vs. 490 m	-0.037	-0.075 to 0.001	0.057	0.018	-0.031 to 0.067	0.466
2590 m vs. 490 m	-0.071	-0.106 to -0.036	<0.001	-0.029	-0.115 to 0.057	0.507
Mean nocturnal oxygen saturation, %	0.007	0.002 to 0.012	0.006	-0.004	-0.015 to 0.008	0.502
Slow wave sleep, min	-0.000	-0.001 to 0.001	0.922			
AHI, 1/h	0.000	-0.001 to 0.002	0.619			
Number of days at altitude						
2nd vs. 1st day	-0.058	-0.087 to -0.029	<0.001	-0.055	-0.085 to -0.026	<0.001
3rd vs. 1st day	-0.088	-0.125 to -0.050	<0.001	-0.085	-0.123 to -0.046	<0.001
4th vs. 1st day	-0.118	-0.159 to -0.077	<0.001	-0.114	-0.156 to -0.072	<0.001
Altitude exposure sequence	-0.008	-0.040 to 0.024	0.633			
Age	0.002	-0.001 to 0.004	0.184	0.002	-0.001 to 0.004	0.253

n=255 observations at 490, 1630 and 2590 m (51 participants). Results are presented for the logarithmically (log 10) transformed tracking error (SD of the mean deviation from the center line) as dependent variable. Independent variables with P<0.2 in the univariable analysis are included into the multivariable analysis. The multivariable model was adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude were 1-4; altitude exposure sequences were A-D, see figure 1. CI=confidence interval; AHI=apnea/hypopnea index.

Table E8. Generalized least square regression analysis of the effect of altitude exposure on the 1, 2, 3 number back test

Dependent variable Log10(mean response time of all correct answers)	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coeffi-cient	95% CI	P
Altitude						
1630 m vs. 490 m	-0.002	-0.027 to 0.023	0.886	0.035	0.006 to 0.064	0.017
2590 m vs. 490 m	-0.011	-0.034 to 0.011	0.316	0.020	-0.137 to 0.054	0.246
Mean nocturnal oxygen saturation, %	0.000	-0.003 to 0.003	0.995			
Slow wave sleep, min	-0.000	-0.001 to -0.000	0.007	-0.000	-0.000 to 0.000	0.124
AHI, 1/h	0.000	-0.000 to 0.001	0.169	0.000	-0.000 to 0.001	0.180
Number of days at altitude						
2^{nd} vs. 1^{st} day	-0.032	-0.046 to -0.017	<0.001	-0.026	-0.041 to -0.010	0.001
3^{rd} vs. 1^{st} day	-0.059	-0.078 to -0.041	<0.001	-0.057	-0.075 to -0.038	<0.001
4^{th} vs. 1^{st} day	-0.077	-0.095 to -0.058	<0.001	-0.071	-0.090 to -0.053	<0.001
Altitude exposure sequence	-0.017	-0.035 to 0.002	0.082	-0.017	-0.034 to 0.000	0.055
Age	0.003	0.001 to 0.004	0.002	0.002	0.001 to 0.004	0.002

n=255 observations at 490, 1630 and 2590 m (51 participants). Results are presented for the logarithmically (log 10) transformed mean response time of all correct answers in the 1, 2, 3 back test as dependent variable. Independent variables with P<0.2 in the univariable analysis and altitude were included into the multivariable analysis. The multivariable model was adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude were 1-4; altitude exposure sequences were A-D, see figure 1. CI=confidence interval; AHI=apnea/hypopnea index

Table E9. Generalized least square regression analysis of the effect of altitude exposure on the trail making test

Dependent variable 1/(time to complete the trail making test)$^{0.5}$	Univariable analysis			Multivariable model		
	Coeffi-cient	95% CI	P	Coef.	95% CI	P
Altitude						
1630 m vs. 490 m	0.004	0.001 to 0.007	0.010	-0.002	-0.005 to 0.001	0.240
2590 m vs. 490 m	0.003	-0.000 to 0.006	0.058	-0.003	-0.006 to 0.001	0.177
Mean nocturnal oxygen saturation, %	-0.000	-0.001 to 0.000	0.918			
Slow wave sleep, min	0.000	0.000 to 0.000	0.006	0.000	-0.000 to 0.000	0.091
AHI, 1/h	-0.000	-0.000 to 0.000	0.231			
Number of days at altitude						
2nd vs. 1st day	0.004	0.002 to 0.005	<0.001	0.003	0.001 to 0.005	<0.001
3rd vs. 1st day	0.009	0.007 to 0.011	<0.001	0.009	0.007 to 0.011	<0.001
4th vs. 1st day	0.012	0.010 to 0.014	<0.001	0.011	0.009 to 0.013	<0.001
Altitude exposure sequence	-0.001	-0.005 to 0.003	0.542			
Age	-0.001	-0.001 to -0.000	0.010	-0.001	-0.001 to -0.000	0.022

n=255 observations at 490, 1630 and 2590 m (51 participants). Results are presented for the reciprocal value of the square root transformed time to complete the trail making test as dependent variable. Independent variables with P<0.2 in the univariable analysis were included into the multivariable analysis. The multivariable model was adjusted for all variables listed. Altitude levels were 1=490 m, 2=1630 m, 3=2590 m; the number of days at altitude was 1-4; altitude exposure sequences were A-D, see figure 1.

CI=confidence interval; AHI=apnea/hypopnea index

Table E10. Pulmonary function tests

	490 m	2590 m		P Friedman ANOVA
		1st day	2nd day	
FVC, % predicted	107 (100;115)	105*(96;112)	103* (98;113)	<0.001
FEV1, % predicted	103 (96;110)	104 (95;111)	103 (95;109)	0.668
FEV1/FVC, %	81 (76;85)	83* (78;87)	82* (78;86)	<0.001
Sniff nasal pressure, cmH_2O	109 (92;129)	108 (87;121)	114 (94;129)	0.062
DLCO adj, % predicted	102 (94;110)	100 (93;107)	100 (91;106)	0.555

Medians (quartiles), n=51. * P<0.05 vs. 490 m (Bonferroni correction by factor 3). No pulmonary function tests were performed at 1630 m. FVC=forced vital capacity, FEV1=forced expiratory volume in one second, DLCO=single breath carbon monoxide diffusing capacity adjusted for barometric pressure.

2.2.2. Ascent to Moderate Altitude Impairs Overnight Memory Improvements

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Abstract

Several studies showed beneficial effects of sleep on memory performance. Slow waves, the electroencephalographic characteristic of deep sleep, reflected on the neuronal level by synchronous slow oscillations, seem crucial for these benefits. Travelling to moderate altitudes decreases deep sleep. In a randomized cross-over design healthy male subjects performed a visuo-motor learning task in Zurich (490m) and at Davos Jakobshorn (2590m) in random order. Memory performance was assessed immediately after learning, before sleep, and in the morning after a night of sleep. Sleep EEG recordings were performed during the nights. Our findings show an altitude induced reduction of sleep dependent memory performance. Moreover, this impaired sleep dependent memory performance was associated with reduced slow wave derived measures of neuronal synchronization. Our results are consistent with a critical role of slow waves for the beneficial effects of sleep on memory that is susceptible to natural environmental influences.

Introduction

Numerous studies have confirmed the beneficial effects of sleep on memory in various tasks (Abel et al., 2013, Rasch and Born, 2013). These studies have demonstrated that the improvement is specifically due to sleep rather than simply due to elapsed time (Abel et al., 2013, Rasch and Born, 2013). Moreover, several studies have linked electroencephalographic (EEG) slow waves, the major electrophysiological characteristic of deep sleep, to such sleep dependent memory improvements. For example, selective slow wave deprivation abolished memory performance improvements after sleep (Aeschbach et al., 2008, Landsness et al., 2009) while boosting slow waves had beneficial effects on memory recall after sleep (Marshall et al., 2006, Ngo et al., 2013).

Sleep at altitude is generally characterized by a shift towards lighter sleep (Nussbaumer-Ochsner and Bloch, 2014), which was recently supported by quantitative and qualitative analysis of the sleep EEG in healthy individuals travelling to moderate altitude (Latshang et al., 2013, Nussbaumer-Ochsner et al., 2012c, Stadelmann et al., 2013). These studies confirmed that sleep at an altitude of 1630 m (and higher) is characterized by a decrease in deep sleep, both measured by a decrease in slow wave sleep (SWS; the deeper non-rapid eye movement (NREM) sleep stages) and a decrease in slow-wave activity (SWA, EEG power between 0.5 and 4.5 Hz) (Latshang et al., 2013, Stadelmann et al., 2013).

It is well accepted that slow waves reflecting deep sleep are essential for the recuperative effect of sleep, i.e. a reduction of sleep pressure (Dijk and Beersma, 1989). On the neuronal level slow waves are reflected by cortical slow oscillations in membrane potential (Steriade, 2000). When these oscillations are near synchronous and involve the majority of cortical neurons, they become evident in the surface EEG as slow waves of large amplitude (Steriade et al., 1993d). Vyazovskiy et al. (2007) has shown that slow oscillations among large populations of cortical neurons are more synchronized at the beginning of a sleep episode compared to the end of sleep. Thus, the reduction of sleep pressure across a night is, on the neuronal level, reflected by a reduction of neuronal synchronization. It has been shown that the slope of slow waves represents a good marker of these

changes in neuronal synchronization: the more synchronized, the steeper are the slopes of slow waves (Esser et al., 2007, Riedner et al., 2007, Vyazovskiy et al., 2007).

Taking advantage of this approach, we calculated the average slope for slow waves of the first and last hour of NREM sleep at two different altitudes, Zurich (490 m) and Jakobshorn (2590 m). Furthermore, memory performance was assessed immediately after learning, before sleep, and in the morning after a night of sleep (see Fig. 1).

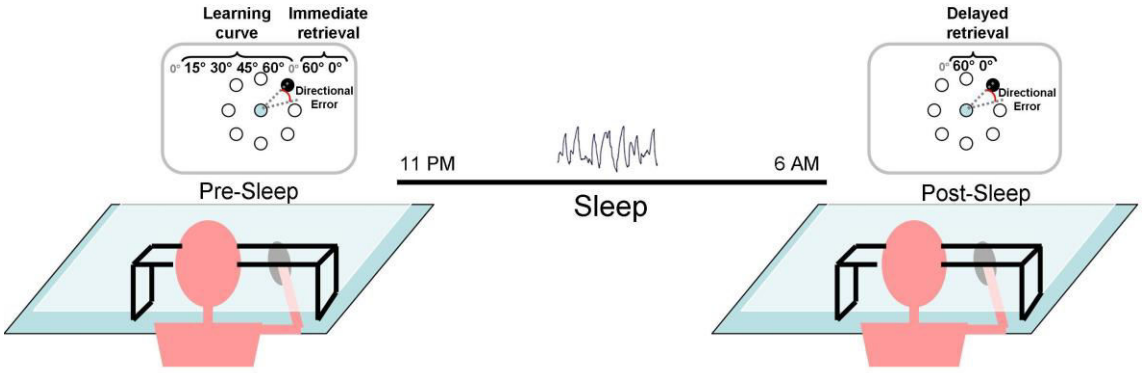

Figure 1: **Study design** - Subjects moved a handheld cursor (mouse) on a digitizing tablet, executing out- and back movements from a central starting point to one of eight targets displayed on a computer screen together with the cursor position. A non-transparent cover prevented subjects from seeing their arm and hand at all times. Unbeknown to the subjects, the cursor position was rotated relative to the hand position by a fixed angle (0° - baseline; 15°, 30°, 45°, 60° - rotation conditions). The subjects learned to perform the task in the evening, adapting stepwise to the imposed rotation, what we call a **learning curve**. Before the last two trial blocks subjects undertook a block of 0° rotation aiming at washing out any residual rotation that was learned previously. Visuo-motor performance (adaptation - 60°) was tested immediately afterwards - after which subjects had to readapt their performance, when the perturbation was removed (0°). After 7 hours of sleep subjects undertook a baseline block after which they were retested in the adaptation condition. Subjects ran through the task sequence twice, once in Zurich at 490 m and once at altitude, at Davos Jakobshorn at 2590 m (within-subject design).

We chose to apply a visuo-motor rotation adaptation task because it permits accurate parameterization of both performance improvement and noise reduction and sleep dependent memory benefits were shown (Huber et al., 2004).

Because millions of visitors travel to moderate altitudes (< 3000m) each year (Hackett and Roach, 2001), be it for professional or personal purposes, the question of how an altitude dependent reduction of deep sleep SWA affects sleep dependent memory improvements becomes a relevant question.

Material and methods

Subjects

Healthy men living below 800 m, 18 to 70 years old, with a body mass index of 18 to 30 kg/m^2 were invited to participate in the study. Subjects with any medical condition requiring treatment, regular use of medications, alcohol, nicotine or drugs, a history of sleep disorders or previous altitude related illness were excluded. Subjects were recruited by advertisements. A stay at altitudes above 1500m during the last 2 weeks before the study period was not allowed. Additionally, the subjects had to abstain from caffeine and alcohol consumption and maintain regular bedtimes (7 h, from 11 p.m. to 6 a.m.) for at least 3 days prior to the recordings. During the study, subjects were asked to stay at current altitude and avoid extensive physical exercise. Compliance was controlled by an actimeter and sleep logs. The study was approved by the local ethics committee and the study was registered (clinicaltrials.gov; ID#NCT01130948). Written informed consent was obtained from all participants and they were monetary compensated upon completion of the study. Forty-nine out of 51 subjects [mean age 27.0 ± 1.3 years (±SEM)] were included for the slow wave slope analysis, of which 26 [mean age 26.9 ± 1.3 years (±SEM)] were included for the analysis of the behavioural task. Two subjects had to be excluded from the slope analysis because of bad sleep quality and thus not enough NREM sleep was available to perform the analysis. Twenty-five subjects had to be excluded from the analysis of the behavioural task due to missing data or technical issues.

Study procedure

The study was performed in the sleep laboratory of the Pulmonary Division of the University Hospital Zurich (baseline, 490 m), at the hospital Davos-Wolfgang (1630 m) and at the hostel Davos Jakobshorn (2590 m), completed in a randomized cross-over design. The protocol consisted of 5 study nights (one baseline night and

two consecutive nights at each higher altitude: 1630 m and 2590 m). We focused our analysis on Zurich (baseline, 490 m) and Davos Jakobshorn (altitude, 2590 m) day/night 1, because we performed our learning task only at these two altitudes. Participants were divided into two groups based on the order of altitude exposure (group 1: within subject design baseline - altitude; group 2: within subject design altitude - baseline). The number of participants was balanced in the two groups for all 49 participants as well as for the subgroup of 26 participants included for the learning task analysis. Subjects had individual bedrooms at all altitudes. Bedtime in the study nights was 7 hours. Lights were turned off at 11 p.m. and switched on at 6 a.m. Every evening and morning, participants completed a test block comprising various medical and cognitive examinations (for more details see (Latshang et al., 2013)).

Polysomnographic recordings

During the seven hours of bed time, EEG (derivations F3A2, F4A1, C3A2, C4A1, O1A2 and O2A1 positioned according to the 10-20 system), submental EMG, EOG and ECG were recorded (Alice5, Respironics AG, Zofingen, Switzerland) together with different respiratory signals (see (Latshang et al., 2013) for details). The EEG data were sampled at 200 Hz (high-pass filter: 0.32 Hz; low-pass filter: 100 Hz; notch filter: 50 Hz).

Sleep stage analysis

Sleep stages (30-s epochs) and arousals were visually scored according to standard criteria (AASM and Force, 1999, Rechtschaffen and Kales, 1968) and visual and semi automatic artefact removal was performed. Because different persons were involved in scoring the sleep stages, all scored nights were reviewed by a single person to assure concordance of the scoring within and between subjects.

EEG Analyses

We used a slow wave detection algorithm similar to the one described by Riedner et al. (2007) (Riedner et al., 2007). Analysis of wave characteristics included NREM sleep stages 2, 3 and 4. Sleep slow waves were identified as negative deflections of the EEG signal (C3A2) between two consecutive zero-crossings. We

only included in our analysis the central derivation because both the frontal and the occipital derivations led to similar results. The negative deflection was chosen due to the higher stability of the signal. However, it has been shown that peak-to-peak analysis shows similar results (Riedner et al., 2007). Only negative half-waves with a frequency between 0.5 and 2 Hz (of any amplitude) were considered for further analysis. From these detected half-waves, we determined the point in time of all zero-crossings and amplitudes (local minima of the signal). The characteristics of slow waves were defined by following parameters: the amplitude, the slope and the incidence of slow waves. The ascending slope of slow waves was defined as the amplitude divided by the time between the point in time of the local minimum and the subsequent zero-crossing. From these detected waves, the mean **amplitude** (μV) and **slope** of slow waves (μV/s) were calculated across the entire night. The **incidence** of slow waves was expressed as number of slow waves detected for the entire night. To control for overnight amplitude differences between the first and the last hour of sleep we calculated the value of regression (i.e., the slope as a function of amplitude) at a fixed amplitude of 75 μV for both time points in Zurich (490 m) and in Jakobshorn (2590 m). This procedure led to one single value for the first and the last hour of NREM sleep, respectively. We chose a fixed amplitude of 75 μV because according to Rechtschaffen and Kales (1968) (Rechtschaffen and Kales, 1968) slow waves are defined as waves with an amplitude of at least 75 μV and it was previously shown that the decrease from early to late sleep was most reliably seen in small amplitude waves (Riedner et al., 2007).

Behavioural task

To investigate performance and overnight performance changes, our subjects performed a visuo-motor learning task just before going to sleep and after waking up the next morning (see Fig. 1). In this task subjects reach for visual targets using a handheld cursor (mouse) while unconsciously adapting to systematic rotations imposed on the perceived cursor trajectory. The motor task required subjects to move the handheld cursor on a digitizing tablet, executing out and back movements from a central starting point to one of eight targets displayed on a computer screen. At target presentation one circle turned black, in synchrony with a tone. At the start of a trial block, subjects positioned the screen cursor within the central start area and a series of three tones followed to provide the required

tempo of the upcoming targets. With the fourth and subsequent tones, successive targets turned black and subjects were instructed to move their hand smoothly out and back to each target without corrections and with sharp reversal. A shield prevented subjects from seeing their arm and hand while performing the task. Unbeknown to the subjects, the cursor position was rotated relative to the hand position by a fixed angle. After 3 baseline blocks, where the subjects got familiarized with the task procedure, there were four incremental steps of 15, up to a maximum of 60 degrees, with three blocks per step, of 88 movements each and with a short break of 1 minute after each incremental step. We computed the directional error for each movement as the angle between the line from the initial hand position to the position of the target and the line to the position of the hand at the peak outward velocity (directional error at peak velocity).

The **learning curve** is represented by the time course of rotation adaptation (incremental steps of 15, up to a maximum of 60 degrees), which was performed only in the evening. To assess the learning curve, directional errors were normalized by the angle of the imposed rotation.

The performance level was tested in separate trial blocks immediately after the learning session (pre-sleep trial block/immediate retrieval) as well as after a night of sleep (post-sleep trial block/delayed retrieval). Before both trial blocks subjects undertook a block of 0 degree rotation aiming at washing out any residual rotation of the just formed internal model/memory. We focused our analysis of visuo-motor performance on 'visuo-motor adaptation' which refers to the subjects' ability to adapt their performance to a visuo-motor perturbation (60 degree rotation - maximal perturbation). As a measure of pre- and post-sleep **final performance level** we calculated the average performance across all 88 movements in the last trial of visuo-motor adaptation - 60 degree rotation (last 88 of the 264 movements) in the evening and in the morning after a night of sleep. Previous studies on visuo-motor adaptation indicated that in addition to the absolute level of performance, the variations in the **dynamics of performance** depend on the level of adaptation: with increasing experience, performance more rapidly reaches asymptotic levels in these kinds of visuo-motor tasks (Debas et al., 2010, Krakauer and Shadmehr, 2006). Therefore, we calculated nonlinear regression models including all 264 movements of the pre- and post-sleep trial blocks (adaptation $f = y + a * exp (-$

*b*x)*). '*b*' was used as a measure for the **speed of adaptation**. For a better representation of the data we multiplied the values of the speed with 10^2.

Statistical analysis

Analysis of the slow wave characteristics were based on data of the conventional derivation C3A2. For the comparison of the slope of slow waves between first and last hour of NREM sleep at the two altitudes a linear mixed model ANOVA was calculated (random intercept: subject (to account for repeated measurements of the same person); main factor: time-interval (first vs. last hour of NREM sleep)). Overnight changes in the slope of slow waves between the two altitudes were calculated by repeated two-way ANOVA with factor time-interval (first vs. last hour of NREM sleep) and factor altitude (Zurich - 490 m, and Davos Jakobshorn - 2590 m). Differences in the learning curves in the evening between the two altitudes were assessed by two-way repeated measure of ANOVA with factor rotation (stepwise increase in the degrees of rotation – 15, 30, 45, 60) and altitude (Zurich - 490 m, and Davos Jakobshorn - 2590 m). Alterations in the speed or final performance level in the behavioural task from 490 m to 2590 m were assessed by 2 (evening vs. morning) x 2 (490 m vs. 2590 m) ANOVAs. In case of significance in the ANOVAs, post hoc paired Student t-tests were performed for further comparisons. For assessing the relationship between different variables we performed Pearson product-moment correlations. Significance was set at 5% level. Throughout the manuscript, data variability is reported as standard errors (mean ± standard errors [SEM]). All analyses were performed using the software package MATLAB (Math Works) and SPSS 16.0.

Results

Learning curve

We first assessed altitude induced changes in the learning curve of the visuo-motor rotation adaptation task, which was assessed in the evening, before the subjects went to bed (pre-sleep) at 490 m, and on the day of arrival at 2590 m. A two-way repeated measure ANOVA (factors altitude (490 m and 2590 m); rotation (raises in rotation in degrees: 15, 30, 45, 60)) revealed that when learning the task in the

evening, our subjects adapted over time to the imposed rotations at both altitudes and did so at the same speed ($F_{rotation}$=15.5, p< 0.01, $F_{rotation\ x\ altitude}$=1.95, p> 0.1).

Altitude dependent overnight changes in performance

In a next step, we investigated overnight changes in final performance level for visuo-motor adaptation to a 60 degree rotation. When comparing the immediate retrieval before sleep (Fig. 2a) and the delayed retrieval after sleep (Fig. 2b) we found a stabilisation at 490 m (evening: 11.4± 0.3; morning: 11.4± 0.3) and a deterioration at 2590 m (evening: 11.6± 0.3; morning: 13.9± 0.3, main effect of timepoint - evening vs. morning: $F_{timepoint}$=3.9, p< 0.05) across the night.

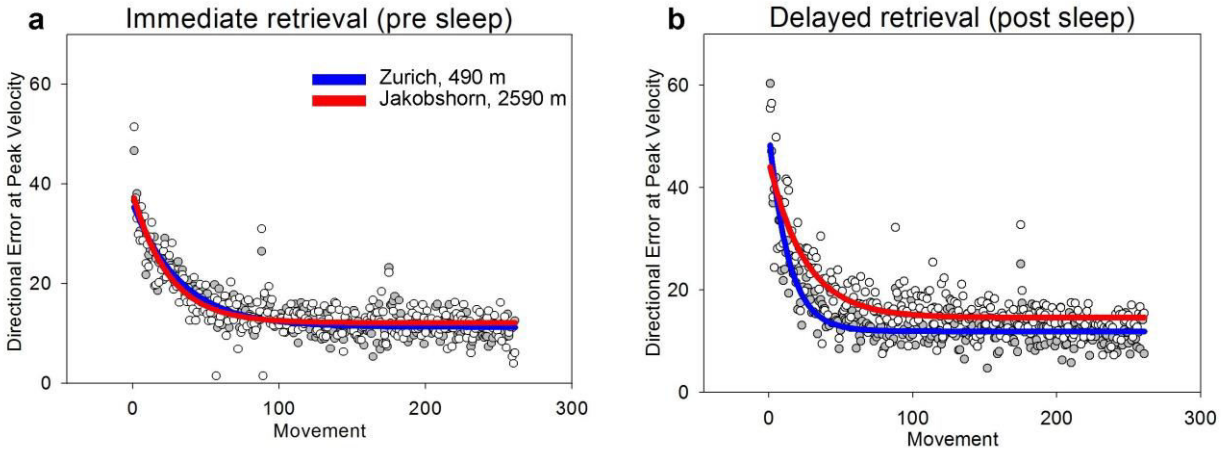

Figure 2: **a, b.** Directional error at peak velocity for the 60° adaptation task (264 movements) in the evening - pre sleep (a) and in the morning after 7 hours of sleep - post sleep (b) for both Zurich (490 m) (filled circles) and Davos Jakobshorn (2590 m) (open circles) (N=26). A curve was fit to the directional error of all 264 movements using nonlinear regression analyses - adaptation: $f = y + a * exp(-b*x)$ for Zurich (490 m) (blue line) and Davos Jakobshorn (2590 m) (red line).

Thus, at 490 m, subjects showed similar directional errors before and after sleep whereas at 2590 m they showed significantly higher directional errors in the morning compared to the evening, thus in the morning there was a significant difference in final performance level between the two altitudes (p< 0.05, paired t-test altitude compared to baseline).

Finally, we also investigated changes in the dynamics of performance, i.e. how fast subjects adapt to the 60 degree rotation. For visuo-motor adaptation in the evening there was no difference between the two altitudes in the speed of reaching their final performance level (Fig. 2a). This adaptation performance (in degrees/s)

improved across the night at 490 m (evening: 4.0± 0.4; morning: 7.6± 1.3; main effect of timepoint - evening vs. morning: $F_{timepoint}$=67.6, p< 0.001) while no such overnight improvement was observed at 2590 m (evening: 4.2± 0.5; morning 4.3± 0.8). As a result, in the morning, subjects showed a significantly lower speed in reaching their final performance level at 2590 m compared to 490 m (p< 0.05, paired t-test altitude compared to baseline) (Fig. 2b).

Altitude dependent changes in the sleep EEG

We then looked at altitude dependent changes in sleep and the sleep EEG. As shown in previous papers reporting results of the same study (Latshang et al., 2013, Stadelmann et al., 2013), exposure to moderate altitude affected sleep architecture. In particular, in our healthy lowlanders, the percentage of SWS (% of total sleep time) showed a 15.8 % reduction at altitude. Total sleep time (TST), NREM sleep, as well as rapid eye movement (REM) sleep were not different between the two altitudes. Our subjects showed a high sleep efficiency both at 490 m and at 2590 m (see Table 1, for more details see also (Latshang et al., 2013) and (Stadelmann et al., 2013).

Sleep variables (mean±SEM)	Zurich (490 m)	Jakobshorn (2590 m)	p-value
TST (min)	392.1±4.1	393.7±3.7	ns
Sleep efficiency (%)	92.9±0.8	92.3±1.1	ns
SWS (%)	24.2±0.9	20.4±0.9	<0.01*
NREM sleep (%)	80.5±0.8	78.2±0.8	ns
REM sleep (%)	19.5±0.8	21.8±0.8	ns

Table 1: Data are provided as means ± SEM (N=49). Total sleep time (TST) in minutes, sleep efficiency in percent (%), slow wave sleep (SWS) in %, non rapid eye movement (NREM) sleep in % and rapid eye movement (REM) sleep in %. * represents one way analysis of variance (ANOVA) < 0.05 (factor altitude: Zurich - 490 m, and Davos Jakobshorn - 2590 m); ns ANOVA > 0.05.

When focussing on slow wave characteristics, we found a significantly lower amplitude of slow waves at altitude, at 2590 m, compared to 490 m. The incidence of slow waves was similar at both altitudes (see Table 2).

Slow wave characteristics (all night)	Zurich (490 m)	Jakobshorn (2590 m)	p-value
Amplitude (μV)	36.2±1.1	33.8±0.9	<0.01*
Incidence (number waves/night)	9905±272.8	9624±272.2	ns

Table 2: Data are provided as means ± SEM (N=49). Amplitude and incidence of slow waves averaged over the entire night (C3/A2). For the analysis, all non rapid eye movement (NREM) sleep episodes (stages 2, 3 and 4) were included. * represents one way ANOVA < 0.05 (factor altitude: Zurich - 490 m, and Davos Jakobshorn - 2590 m); ns ANOVA > 0.05.

When plotting the slope of slow waves against their amplitude (in 10μV bins) a significant decrease in the slope of slow waves from the first to the last hour of NREM sleep was observed over the entire amplitude range for both altitudes (Fig. 3a and 3b). To control for amplitude differences between the first and the last hour of sleep we calculated the slope at a fixed amplitude of 75 μV for both time intervals (first vs. last hour of NREM sleep) and altitudes (490 m vs. 2590 m) separately. We found a significant decrease in the slope from the first to the last hour of NREM sleep both at 490 m as well as at 2590 m (Fig. 3c). However, at 2590 m, the difference in the slope from the first to the last hour of NREM sleep was significantly reduced compared to 490 m (Fig. 3c inset). This reduced difference was mainly due to significantly reduced slopes in the first hour of NREM sleep at 2590 m compared to 490 m (Fig. 3c).

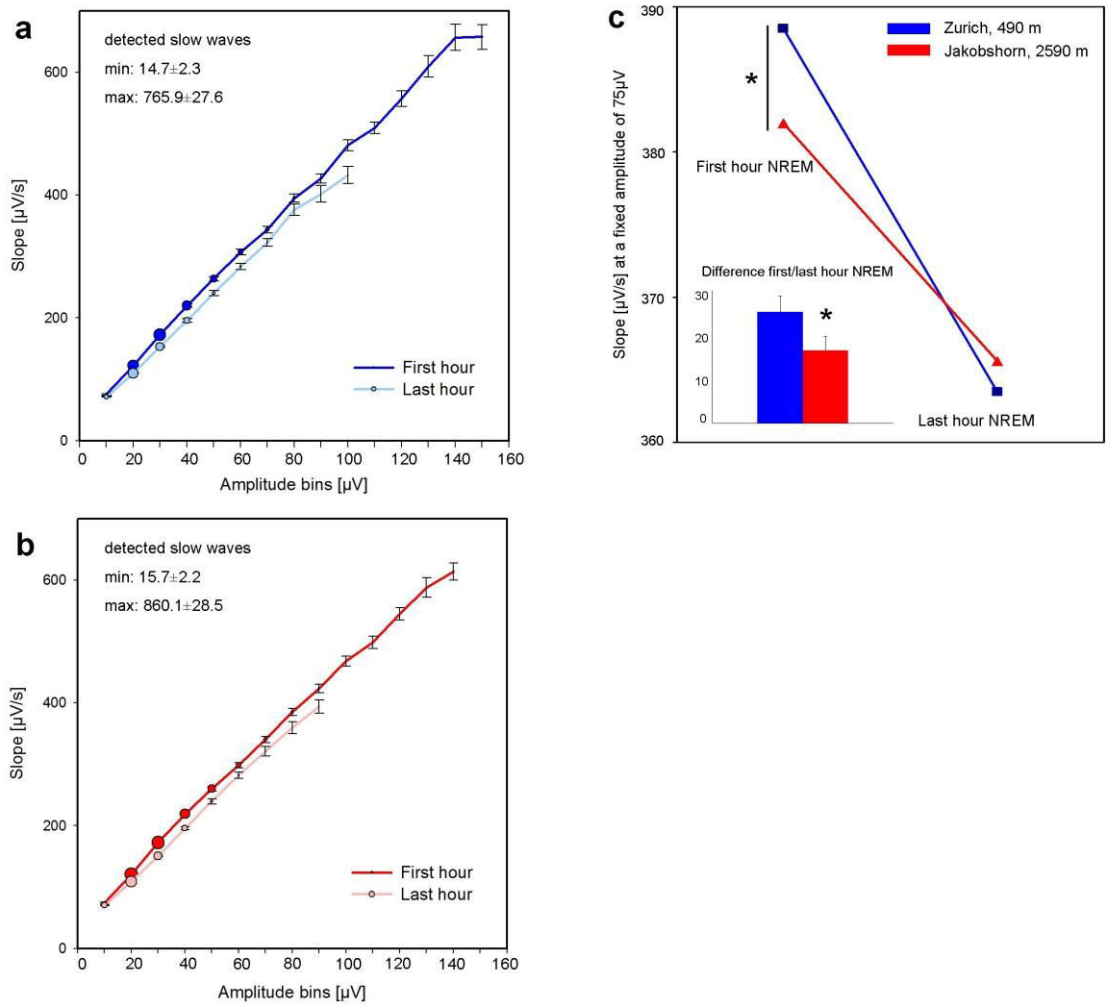

Figure 3: **a, b**. Slope of slow waves for consecutive 10µV amplitude bins of the first and last hour of NREM sleep (C3/A2) (mean±SEM) for Zurich (490 m) (a) and Davos Jakobshorn (2590 m) (b) (N=49). Only amplitude bins with at least 15 detected waves per subject are considered. Circle size represents the number of detected slow waves for each amplitude bin. Numbers in the upper left corner show the maximal and minimal number of detected slow waves across all subjects (mean±SEM). A decrease in the slope from the first to the last hour of NREM sleep was observed for the entire amplitude range at each altitude. A linear mixed model ANOVA was calculated for each altitude separately (random intercept: subject (to account for repeated measurements of the same person); main factor: time-interval (first vs. last hour NREM sleep) Zurich (490 m): $F_{time\text{-}interval}$=170, p< 0.001; Davos Jakobshorn (2590 m): $F_{time\text{-}interval}$=135.7, p< 0.001). **c.** Overnight changes of the slope from the first to the last hour of NREM sleep. Mean±SEM of the slopes at an amplitude of 75µV are shown for both altitudes. A repeated two-way ANOVA revealed significant effects for the factor time-interval (first vs. last hour NREM sleep) ($F_{time\text{-}interval}$=54.4, p< 0.001) and for the interaction time-interval (first vs. last hour NREM sleep) x altitude (Zurich - 490 m, Davos Jakobshorn - 2590 m) ($F_{altitude\ x\ time\text{-}interval}$=5.3, p< 0.05). The differences in the first hour and in the last hour between ZH and JH were compared separately by paired t-tests. **Inset***:* Bars represent overnight differences of the slope from the first to the last hour of NREM sleep. Mean±SEM of this

difference at an amplitude of 75µV are shown for both altitudes. Differences between the two altitudes were compared using paired t-test. * $p < 0.05$ indicate significant differences.

Correlation between sleep EEG and performance changes

Finally, we performed correlational analysis to address the question whether a relationship between the reduction in the slope of slow waves and overnight performance changes exists. We focussed on the difference in overnight performance in the speed of reaching the final performance level when adapting to the 60 degree rotation between the two altitudes, since this variable showed a clear overnight performance improvement at 490 m compared to 2590 m. Our results revealed a positive correlation between this performance change and the slope difference between the first and the last hour of NREM sleep when comparing the two altitudes ($R=0.6$, $p < 0.01$, Pearson product-moment correlation).

Discussion

Our results showed that an ascent to a moderate altitude has a negative impact on sleep dependent memory improvements. Thus, we identify yet another physiologic parameter, which is affected by altitude and may impact performance at altitude.

It is well known that ascending to higher altitudes can cause mountain sickness, an acute syndrome, associated with an inadequate adaptation to a hypoxic environment (Virues-Ortega et al., 2004). The symptoms are often unspecific, the most characteristic ones being headache, insomnia and fatigue and they appear in up to 9% of people who have just reached an altitude of 2850 m or more (Maggiorini et al., 1990). None of our subjects experienced signs of acute mountain sickness. However, during sleep at altitude, subjects showed a reduced oxygen saturation level (SpO2) and a periodic breathing pattern with increased central apneas/hypopneas (AHI) associated with oxygen desaturations (ODI) (Latshang et al., 2013). Thus, a disturbed breathing related reduction in deep sleep, as shown previously (Khoo et al., 1982, Latshang et al., 2013, Nussbaumer-Ochsner et al., 2012a, Stadelmann et al., 2013) might have resulted in a negative impact on sleep dependent memory improvements. However, correlational analysis showed no evidence that the altered breathing pattern accounted for the observed reduced sleep dependent memory improvement (data not shown).

We found a positive association between our measure of neuronal synchronization and the overnight memory performance improvement. This result allows some speculations about underlying mechanisms of the relationship between deep sleep and memory performance improvements. The synaptic homeostasis hypothesis proposes that a progressive reduction of synaptic strength across sleep due to downscaling is tied to the beneficial effects of sleep on performance (Diekelmann and Born, 2010, Nere et al., 2013, Tononi and Cirelli, 2003). Such a reduction in synaptic strength is reflected in a reduction of synchronization of neuronal activity. The slope of slow waves is a reflection of how fast neuronal populations can synchronize their activity and therefore indirectly reflects the decrease of synaptic strength across the night (Esser et al., 2007, Riedner et al., 2007, Vyazovskiy et al., 2009). Accordingly, in humans and rats it was shown that the slope of slow waves decreases across the night (Fattinger et al., 2014, Kurth et al., 2010a, Riedner et al., 2007, Vyazovskiy et al., 2009) presumably reflecting the downscaling of synaptic strength across the night. The present results show that it is specifically the overnight reduction in the slope of slow waves, which is related to overnight performance changes since no such correlations were found for the incidence and the amplitude of slow waves (data not shown).

The question remains what might have triggered these changes in neuronal synchronization at altitude? One possible explanation might be that exposure to mild hypoxia enhances sympathetic activity and catecholamine levels (Heistad and Abboud, 1980, Kelly et al., 2010, Rowell et al., 1986, Selvamurthy et al., 1981, Stowhas et al., 2013). Interestingly, of all the physiological variables we tested (breathing parameters such as SpO2, AHI and ODI, blood pressure and heart rate) (for more details see (Latshang et al., 2013, Stowhas et al., 2013)) heart rate was the only parameter that showed a negative association with the slope of slow waves, our measure of neuronal synchronization ($R=-0.49$, $p< 0.01$, Pearson product-moment correlation). Since heart rate is under autonomic control this correlation might indicate a relationship between neuronal synchronization and sympathetic activity. Direct manipulations of sympathetic activity might prove such a relationship. The secretion of cortisol is also sympathetically regulated (van Stegeren et al., 2007) and correspondingly was shown to increase with a rise in altitude (Moncloa et al., 1967). Furthermore, increased levels of cortisol impaired performance when cortisol was administered during sleep (Wagner and Born,

2008). Thus, an altitude dependent elevation of the cortisol level might have reduced the beneficial effects of sleep on memory performance. Whether this effect of cortisol on sleep dependent memory improvement is mediated via a change in the level of synchronization needs to be experimentally tested.

In summary, our results are consistent with an adverse effect of the hypoxic environment at altitude on sleep related memory consolidation mediated by a reduction in slow waves. In other words, when travelling to moderate altitude, an important function of sleep in synaptic homeostasis seems to be impaired with negative consequences on sleep dependent memory performance. These findings may have important implications for a large number of people travelling to altitude, including further environments at which barometric pressures are similar to those at terrestrial altitudes, as for example aircraft-cabins (Muhm et al., 2009).

3

General Discussion

The studies enclosed in the present thesis support a close relationship between specific sleep EEG characteristics - slow waves and sleep spindles - and cortical plasticity 1) during a vulnerable developmental period in both health and disease and 2) in the context of natural environmental influences.

The REVIEW article introduced sleep during development and shed light on the interplay between both sleep structure and sleep EEG characteristics and healthy development as well as early onset psychiatric diseases. In the first part of the thesis, we investigated possible alterations in sleep EEG characteristics in early onset psychiatric diseases by means of high density EEG, which offers a unique opportunity to combine the temporal resolution of the EEG with high spatial resolution. More specifically, we looked into potential changes in sleep spindles in adolescents diagnosed with Early Onset Schizophrenia (1) as well as into possible alterations in the topography of sleep SWA in adolescents diagnosed with Major Depression (2). Hereby, we were able to detect early neurodevelopmental deviations in both disease groups, such as a deficit of spindles in youth with schizophrenia and increased frontal SWA in depressed youth. In a third approach, we were interested in sleep and plastic changes in healthy development. Therefore, we investigated individual changes in SWA during childhood and adolescence longitudinally and related possible alterations of SWA to performance in a specific visuo-motor task (3). Our data convincingly shows that SWA topography is a trait that can be tracked within individuals longitudinally and differs between individuals. Furthermore, our findings support that sleep SWA can be used as a mirror for motor skill development and cortical restructuring during adolescence.

In the second part of the thesis, we investigated sleep and cognitive performance at moderate altitude in healthy adults (4). We indeed found alterations in sleep structure, together with breathing disturbances due to a moderate increase in altitude. However, these alterations did not affect daytime cognitive performance. We therefore further investigated whether a moderate increase in altitude affected neuronal synchronization - the slope of slow waves - and thus overnight changes in memory performance by using the same visuo-motor task applied in study 3 (5). We chose to apply this task because it permits accurate parameterization of both performance improvement and noise reduction. Also sleep dependent memory

benefits were shown in the past (Huber et al., 2004). We thereby investigated whether sleep dependent memory benefits in adults are affected by a moderate increase in altitude. We indeed found an altitude induced reduction of sleep dependent memory performance. Our results are consistent with a critical role of slow waves for the beneficial effects of sleep on memory that is susceptible to natural environmental influences.

In the next sections, I will discuss the results of the thesis and address some possible future research aims by first focusing on sleep during development and in the context of early onset psychiatric diseases and secondly on sleep and plasticity in the context of natural environmental influences.

3.1. Sleep during development and in the context of early onset psychiatric diseases: Discussion of findings and outlook

Across cultures, adolescence has been observed as a time of dramatic modifications in both body and behaviour. While most teenagers successfully complete the transition from dependence to being an independent member of society, adolescence is also a time of increasing incidence of several mental diseases, including affective and psychotic disorders (Paus et al., 2008). Amongst others, the pathophysiology of these diseases is understood as arising from maturational aberrations in the adolescent brain. It has been suggested that suboptimal timing and magnitude of alterations occurring in adolescent neural systems increases the risk of early onset psychiatric disorders (Tesler et al., 2013). Understanding the basis of these disorders requires a comprehensive knowledge of how the brain works. Many advances can be stated but still a lot remains unclear. The current thesis provides a further step towards the rather challenging task to better understand and recognize early onset psychiatric diseases. In the subsequent lines, I will discuss our results in the context of future research.

Focus on sleep spindles

Recent studies reported a critical role of the thalamic reticular nucleus (TRN) in generating sleep spindles as well as in processing sensory information (Ferrarelli and Tononi, 2011). Adult patients with schizophrenia show marked spindle deficits (Ferrarelli et al., 2007; Ferrarelli et al., 2010; Wamsley et al., 2012). These findings contributed to a better understanding of certain aspects of the phenomenology and neurobiology of schizophrenia. Additionally, they seem of clinical significance since in first analyses the spindle deficits could be successfully used to differentiate patients affected by schizophrenia from other patients or healthy controls (Ferrarelli et al., 2007). However, for an adequate comprehension of disease development, studying patients with Early Onset Schizophrenia (EOS) is fundamental and it remains to be shown if developmental aspects influencing brain maturation as well as symptom expression during disease further interact with spindle characteristics during sleep. So far, no study investigated alterations in sleep spindles in EOS patients. Our results, including the negative associations between positive symptoms of schizophrenia and sleep spindles, replicate the findings seen in adults, indicating that already children and adolescents with this disorder show such deficits. At this stage, we can only speculate about the contribution of TRN deficits to the positive symptoms of schizophrenia, including hallucinations and delusions. A reduced inhibitory control of the TRN on other thalamic nuclei would increase the response of thalamocortical neurons. This may facilitate inner sensory information to overwhelm cortical regions and result in strong perceptions in absence of external stimuli - hallucinations. Next to hallucinations, patients with schizophrenia commonly experience delusional thoughts. Such thoughts tend to arise in a state of hypervigilance, characterised by increased neuronal activity and thus enhanced response to incoming stimuli. It is therefore possible that a reduced inhibitory control of the TRN on incoming stimuli may result in a hyperactivation of the cortex, which, consecutively, may produce delusions (Ferrarelli and Tononi, 2011). In addition to hallucinations and delusions, patients with schizophrenia also report cognitive deficits (Gur et al., 2007). In line with previous literature, cognitive performance is lower in EOS patients compared to healthy controls (Ferrarelli et al., 2010, Green et al., 2004). While many factors, including changes in the level of attention, motivation and the presence of diverse symptoms may affect cognitive processes, it is

interesting that such processes are regulated during sleep by sleep-specific rhythms, including spindles (Fogel et al., 2007). Higher spindle activity has been repeatedly associated with better performance in healthy individuals (Fogel et al., 2007; Rasch and Born, 2013). A defective TRN function, which sets off spindle oscillations, may then interfere with the skill to process information and may thus further account for some of the cognitive deficits found in schizophrenia (Ferrarelli and Tononi, 2011, Wamsley et al., 2012). However, in contrast to slow waves, where by means of experimental manipulations, memory performance could be enhanced (Marshall et al., 2006, Ngo et al., 2013), so far there is no evidence for a causal relationship between sleep spindles and cognitive performance. Future studies exploring the relationship between spindle deficits and specific cognitive domains in schizophrenia may provide further insight into the neural circuits underlying such impairments. Altogether, our results helped to underline the close relationship between sleep spindles and positive symptoms of schizophrenia and further point to the key role of TRN within the neurobiology of this disorder. The diagnostic process is challenging as relying on reported symptoms alone, the disorder often remains undetected, especially in children and adolescents, where the symptom expression is less overt. However, early treatment of schizophrenia was shown to ameliorate outcome, which further stresses the importance to further elucidate whether spindle deficits may serve as an electrophysiological marker to facilitate the diagnostic process, irrespective of age of onset.

Focus on slow waves

In our first study, the spotlight was on sleep spindles, because previous studies consistently reported sleep spindle deficits in adult schizophrenia patients. In contrast to reduced sleep spindles, slow wave parameters did not differ in adult schizophrenia patients (Ferrarelli et al., 2010). Slow waves are generated by cortico-cortical connections, although thalamocortical neurons also contribute to the synchronous onset of cortical firing (Crunelli and Hughes, 2010). SWA has been scantily investigated in schizophrenia patients, however, so far, with conflicting results. Two studies did not report differences (Ferrarelli et al., 2007; Goder et al., 2006) while three showed reduced SWA in schizophrenia patients (Hiatt et al., 1985; Keshavan et al., 1998). However, these results were found in adults and so far,

there is no study looking into SWA alterations in EOS. Feinberg (Feinberg, 1982) speculated that schizophrenia might be a consequence of an exaggeration of the typical synaptic elimination that takes place during adolescence. According to the synaptic homeostasis hypothesis (SHH), sleep slow waves may actively contribute to cortical maturation (Tononi and Cirelli, 2006), especially during the massive changes and dynamics of brain maturation taking place in childhood and adolescence (Feinberg and Campbell, 2010a, Feinberg and Campbell, 2013). Therefore, we were further interested in the SWA topography in our young EOS sample. Preliminary results revealed that compared to age- and gender-matched healthy controls, SWA was significantly increased over the left temporal lobe in our EOS sample (Figure 1).

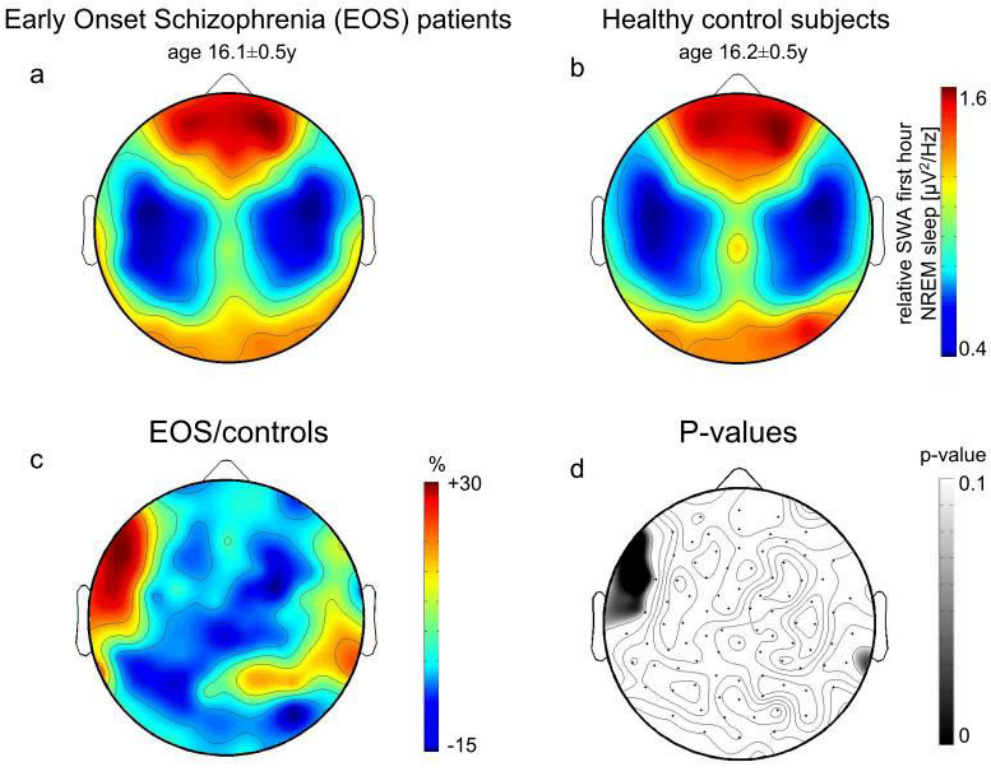

Figure 1: Topographical distribution of SWA (1-4.5 Hz) for the first hour of NREM sleep stages 2 and 3 in EOS patients (a) and healthy, age- and gender-matched control subjects (b). Values are colour coded (maxima in red, minima in blue) and plotted on the planar projection of the hemispheric scalp model. c, d. Topographical distribution of the difference in SWA between EOS patients and control subjects (ratio EOS/controls). Values are colour coded (group differences in %). SWA was increased in a cluster of left temporal electrodes (c), with p-values indicated in (d).

Temporal lobe structures have been repeatedly shown to be involved in hallucinations (Barta et al., 1990; Palaniyappan et al., 2012) and a recent study reported abnormalities of grey and white matter volumes in the temporal lobes in children with first-episode schizophrenia as well as in the prodromal phase of the illness (Cullen et al., 2013). Our preliminary results point towards an involvement of SWA in use-dependent plasticity processes in EOS patients. Increased SWA in our patients may reflect increased use of networks in this region during daytime, as most of our patients may have experienced hallucinations. According to the SHH, hallucinations during wakefulness may have increased synaptic strength in the temporal lobes and increased SWA would be the response during night-time. However, in the context of a disorder and this amount and intensity of misinformation during daytime, the intrinsic homeostatic balance may not be sufficient. Even worse, downscaling may hit the previously "healthy" synapses and the strengths of their connection may be weakened or potentiation might occur, reinforcing or stabilizing the "wrong" circuits and pave the way for hallucinations. In this sense, hallucinations may be partially learned and consolidated during the night. Future investigations should increasingly focus on alterations in SWA topography in EOS with regard to local as well as global effects and consider the initial stages of NREM sleep as well as the course of the night to observe stability of observed patterns. Not only harming events or misinformation but also helpful interventions and specific cognitive trainings may profit from the plasticity of the developing brain (Tesler et al., 2013).

Maturational trajectories or use-dependent dynamics - or an interaction of both?

As SWA offers a unique advantage of mapping anatomical and functional alterations, in our second study, we investigated the topography of SWA in adolescents diagnosed with Major Depression. The comparison of the SWA topography between healthy controls and adolescents diagnosed with Major Depression showed a local increase of SWA over frontal brain regions in the latter group. According to the SHH (Tononi and Cirelli, 2006) and based on numerous studies confirming a close relationship between synaptic changes and SWA (Esser et al., 2007; Vyazovskiy et al., 2008; Vyazovskiy et al., 2009) we have interpreted these findings as reflecting group differences in the underlying functions. So far, in

the literature, differences in SWA topography were clearly assigned to either structural (maturational) or functional (use-dependent) origin (Finelli et al., 2001; Kattler et al., 1994; Kurth et al., 2010). Thereby a clear distinction was possible, because the study participants were either healthy adults where the observed differences could be attributed to the applied experimental manipulation, or healthy maturing children of different ages, where the detected alterations were most probably age-dependent and thus of maturational origin. In contrast, in our study, the subjects were developing children and adolescents, who were diagnosed with a psychiatric disorder. How can in this case maturational long-term aspects and functional dynamic short-term effects be disentangled? Although an impact of each of these processes is plausible independently, a combination of both mechanisms seems likely. Hereby, frequently increased daytime use of specific brain regions, during a period where major neurobiological modifications occur, could alter the time course of typical healthy cortical restructuring. In the subsequent lines, I would like to discuss this interplay.

Neuronal development involves competing processes of formation and elimination of neuronal branches and it has been suggested that personal experience may play a major role in these processes. Early work by Hubel and Wiesel demonstrated that adequate stimulation during a crucial period is needed, to establish a properly functioning architecture of the visual system, further determining the final outcome without a later possibility of correction (Wiesel and Hubel, 1963). This may be an extreme example of a specific interplay within a particular sensory system and the brain may well be much more plastic, however, it shows that experience of light is vital for a normal development of the visual system. The interplay between biological and environmental influences has been described as 'experience-expected' versus 'experience-dependent' mechanisms (Galvan, 2010). 'Experience-expected' suggests that a certain experience is expected to occur roughly at the same time point during development in all humans (i.e. visual or auditory input begins shortly after birth). On the other hand, 'experience-dependent' processes describe individual opportunities for specific experiences. A consequence of experience was also observed on the molecular level. It was shown that the intensity of stimulation induces changes in the number of synapses: for example, an increase in synapse number and dendritic branching was observed after being exposed to an enriched

environment, while sensory deprivation decreased synapse number (Fiala et al., 1978; Greenough et al., 1973; Knott et al., 2002).

Such experience driven plasticity was also shown for sleep: raising mice or cats after birth in absolute darkness resulted in a strong reduction of SWA in the primary visual cortex which recovered gradually after re-exposure to light (Toyoizumi et al., 2013). In contrast, keeping mature animals in complete darkness did not affect SWA (Miyamoto et al., 2003) while another study confirmed the finding that light deprivation well after the critical period of ocular dominance plasticity has little effect on receptive field properties (Reid and Daw, 1995). Consequently, sleep, specifically SWA, seems to play an essential role during development.

Taken together, timing and experience play an important role in brain development. It can further be speculated that during early childhood, experience-expected processes contribute most to the refinement of cortical areas. Vision, auditory and somatic sensory systems are shaped and depend on external information in a specific time-frame. Later on, improved skills allow the individual to be actively involved in daily life experiences while the maturing brain is additionally challenged by different demands such as social interaction and formation of a self-concept. Diverse experience-dependent situations may occur, while further enhancing processes of brain plasticity by overwhelming compensatory mechanisms. Sleep, specifically SWA, seems to have a major role in regulating these processes (Wilhelm et al., 2014). Some experiences appear to contribute to the variability between humans, however, other experiences may be vital for proper development (Paus et al., 2008). These findings point towards the dynamic nature of brain development and hereby for the high vulnerability for the onset of psychopathologies during development, especially in the phase of adolescence (Paus et al., 2008). However, at this point, we are not able to disentangle between experience-expected and experience-dependent plasticity processes. Nevertheless, the use of SWA topography by means of hdEEG may bridge this gap and display maturational as well as functional alterations. Furthermore, SWA topography may guide research a step closer towards the rather challenging mission to better understand and recognize early onset psychiatric diseases. Longitudinal studies with the simultaneous detection of impeding symptoms and electrophysiological parameters

may help to further disentangle the underlying processes and follow changes in SWA topography on an individual level. Preliminary analyses in this regard illustrate intraindividual SWA alterations over time along with improved symptom severity in one of our participants, initially diagnosed with Major Depression (Figure 2). Also, longitudinal monitoring of changes in SWA may elucidate the timing of the disorder, because the trajectory of cortical maturation has been shown to be a better predictor for future outcome (Shaw et al., 2008). Understanding how SWA contributes to normal and abnormal brain development may identify disturbed mechanisms underlying a disorder and offer potential interventions. As our approach proves to be promising, following individuals longitudinally would be the next step to pursue.

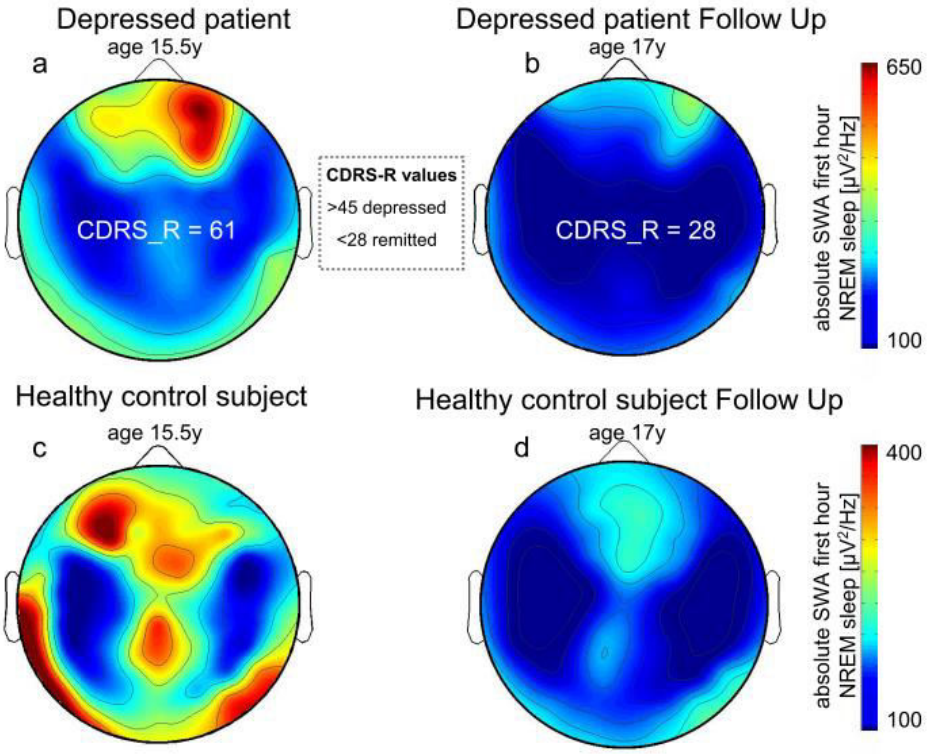

Figure 2: Topographical distribution of SWA (1-4.5 Hz) for the first hour of NREM sleep stages 2 and 3 in a depressed subject timepoint 1 (a) and timepoint 2, follow up (b), together with the indication of alterations in the Children's Depression Rating Scale Revised (CDRS-R) symptom score. (c) and (d) indicate the topographical distribution of SWA in a healthy control subject timepoint 1 and timepoint 2, follow up. Values are colour coded (maxima in red, minima in blue) and plotted on the planar projection of the hemispheric scalp model.

However, before being able to draw conclusions from such longitudinal studies, understanding healthy intraindividual development is fundamental. Therefore, in our third study, we examined SWA topography in healthy adolescents and revealed a strikingly stable pattern. Previously, Tarokh and colleagues (Tarokh et al., 2011) reported that the morphology of the sleep EEG spectrum is largely preserved between repeated measures in adolescents. Thereby, the sleep EEG seems to be highly determined by the genetic background, for which evidence is provided in twin studies (De Gennaro et al., 2008; Landolt, 2011). We added to this finding an important contribution, showing that SWA topography is highly preserved across adolescence. Consequently, SWA topography may represent a valuable biological endophenotype even in periods of pronounced cortical restructuring (Tarokh et al., 2011). Not only sleep EEG in general but also SWA topography in particular may be among the strongest heritable traits in humans.

Familial and genetic risk

Offspring of parents with Major Depression face three to four times increased rates of depression compared to offspring of healthy parents (Rice et al., 2002). Our results of a similar SWA topography in both our depressed sample and their healthy siblings underline the importance of vulnerability due to heritability or the common environment, or the interplay of both, in early onset psychiatric disorders. Future studies may intensify research in individuals at genetic risk and should also carefully screen for family history of psychiatric diseases in healthy controls. According to our results, we would expect a positive association between increased frontal SWA topography not only in depressed individuals and their siblings but also in our sample of healthy controls with a family history of a depressive disorder.

Comorbidities and symptom magnitude

Family and twin studies suggest that anxiety and depression share inherited liability, but anxiety in childhood tends to precede later depression during adolescence (Thapar and Rice, 2006). A possible approach for future studies would be to examine possible mechanisms whereby anxiety serves as a precursor to depression in adolescents. One possibility is that depression and anxiety are actually distinct

processes, but the experience of anxiety at one age predisposes the individual for later depression (Rice et al., 2002). For example, separation anxiety, social phobia and agoraphobia can impair social and academic functioning. Children with such anxieties are less apt to experience the rewards of social or academic success during a critical time, where self-concept development is taking place (Cole et al., 1998). Furthermore, next to anxiety disorders being a precursor of depression, negative, depressive symptoms in schizophrenia seem to precede the onset of positive symptoms (Rutter et al., 2006). Therefore, studies in individuals at genetic risk as well as in individuals symptomatically at risk may further disentangle the progression of symptomatology and interaction with SWA topography. Our results of a positive association between frontal SWA and the difference between subjective and objective sleep latency in a sample of 31 healthy adolescents and young adults support the notion that negative biases and altered memories possibly exist as part of a continuum and depend on the same neurobiological system as full blown symptoms. This finding supports the idea that abnormalities occur in a graded fashion, varying in quality and quantity, where deviations from normality can not only be either present or absent, but exist as part of a continuum (Kendell, 1969; Merikangas et al., 2003). Thereby genes and behaviour may not be associated on a crude, one-to-one basis; the relationship between a gene and a behaviour is probably more comparable to chaos theory's 'sensitive dependence on initial conditions' (Hasler et al., 2004). For example, there is most probably no gene for 'language' alone; there are several genes that mould the embryonic brain in such a way as to ease the physiological processes required for language learning. In a comparable way, no single gene has been found to code for a particular human psychiatric condition. It is rather the interaction of vulnerability genes with environmental factors leading to the development of normal and abnormal human behaviour (Glazier et al., 2002; Petronis, 2001).

Taken together, SWA topography seems to be a reliable mapping tool that mirrors and/or precedes disturbed processes of cortical brain plasticity. Furthermore, SWA might represent a promising endophenotype which has the potential to identify subtle abnormalities not only in early-onset depression but already in adolescents at risk as well as in healthy individuals. The perceptual disconnection from the environment during sleep might be a further advantage of hdEEG compared to other

imaging techniques and particularly relevant for studies in children with psychiatric diseases.

In the long term, the development of a new diagnostic system that includes reported symptoms as well as biological endophenotypes will enhance early recognition and treatment of psychiatric disorders and may contribute to the dimensional approach of these disorders rather than the categorical classification, since a reduction of phenotypic heterogeneity seems crucial for the identification of vulnerability factors.

3.2. Sleep and plasticity in the context of natural environmental influences: discussion of findings and outlook

Our randomized crossover trial in a large sample of healthy young men living near sea level revealed a substantial and individually highly variable amount of sleep related periodic breathing associated with mild hypoxemia during acute exposure to altitudes of 1.630 m and 2.590 m. We found mild alterations in sleep structure and in subjective sleep quality. However, the results revealed that the impact of moderate altitude on breathing disturbances, causing the observed alterations in sleep architecture were not severe enough to significantly affect any cognitive daytime measures in healthy young subjects. Nevertheless, this finding might be different at higher altitudes or in an older population where altitude-related breathing disturbances are more severe (Kohler et al., 2008). As part of this study, a recent investigation additionally found a considerable decline in SWA in an altitude-dependent manner (Stadelmann et al., 2013). In our last study, we found a reduction in the amplitude and slope of slow waves at moderate altitude, 2.590 m. However, the incidence of slow waves did not change with an increase in altitude. These findings provide further evidence that hypoxia affects sleep EEG characteristics already at more moderate altitudes. On the cortical level, the high amplitude low-frequency EEG, detected during deep NREM sleep, is associated with highly synchronized cortical activity. Furthermore, multiunit studies revealed that slow waves are generated by large populations of neurons displaying synchronous on and off states (Timofeev et al., 2001, Vyazovskiy et al., 2009). On the single neuron level, up-states are associated with membrane depolarization and down states with

a hyperpolarization of the cell (Vyazovskiy and Harris, 2013). One possible mechanism to reduce slow waves would be to inhibit or disturb the process of neuronal synchronization. Thereby, respiratory disturbances or other external stimuli could lead to a reduction of slow waves (Dijk, 2010). As a consequence, on the scalp EEG level, a reduction in the synchrony of cortical neurons would be observed which could be further explained by a decrease in the amplitude of slow waves (Vyazovskiy et al., 2009). A concurrent decrease in the amount of slow wave sleep is not compulsory, as the proposed mechanism would mainly affect the amplitude, not the incidence of slow waves.

A number of studies have confirmed the beneficial effects of sleep on memory in various tasks (Abel et al., 2013, Rasch and Born, 2013). These studies have demonstrated that the improvement is due to sleep, not only due to passage of time (Abel et al., 2013, Rasch and Born, 2013). Furthermore, these studies have linked sleep slow waves to such sleep dependent memory improvements. For example, boosting slow waves had beneficial effects on memory recall after sleep while selective slow wave deprivation abolished memory performance improvements (Landsness et al., 2009, Marshall et al., 2006, Ngo et al., 2013). Therefore, in a next step we further addressed the question as to whether the well documented beneficial effects of sleep on memory (Abel et al., 2013, Rasch and Born, 2013) are altered by a moderate increase in altitude and how these findings improve our understanding of mechanisms underlying the relationship between sleep and memory. We investigated how overnight performance changes were affected by a moderate increase in altitude and whether possible alterations were related to changes in neuronal synchronization - the slope of slow waves, which represents a good marker of these changes in neuronal synchronization. We indeed found a reduction in neuronal synchronization, along with a reduction in overnight memory performance. Thus, we added another parameter which was affected by altitude and may impact performance at altitude.

Increased sympathetic activity at altitude?

It is well known that ascending to altitude can cause mountain sickness, an acute syndrome, associated with a poor adaptation to a hypoxic environment (Virues-

Ortega et al., 2004). The symptoms are often unspecific, the most typical ones being insomnia, headache and fatigue (Virues-Ortega et al., 2004). None of our subjects experienced such symptoms. However, they showed breathing disturbances as well as slight alterations in sleep architecture. Correlational analyses showed no evidence of an association between breathing disturbances and our finding of reduced neuronal synchronization and overnight memory improvement at moderate altitude. We therefore attempted to find another factor that could have affected our findings. As part of the same study, Stowhas and colleagues could show that heart rate increased significantly at altitude (Stowhas et al., 2013). Interestingly, we also found an association between our measure of neuronal synchronization and heart rate. Why would heart rate have an effect on our findings? Heart rate is under autonomic control and an activation of the sympathetic activity could lead to the observed findings. To follow this issue, we further investigated alterations in the slope of slow waves at moderate altitude in a group of 30 patients with Obstructive Sleep Apnea (OSA), both under placebo condition as well as under treatment with acetazolamide, which is a medication, known to alleviate altitude dependent oxygen desaturation level and acute mountain sickness (Nussbaumer-Ochnser et al., 2012; Leaf and Goldfarb, 2007). Compared to Zurich, at Jakobshorn, the patients showed alterations in the overnight change in the slope of slow waves in both conditions, under placebo condition and when treated with acetazolamide. Furthermore, the patients under treatment with acetazolamide showed an increase in the slope of slow waves from the first to the last hour of NREM sleep (Figure 3). Consequently, it seems that some physiological changes related to an increase in altitude led to the observed alterations in the slope of slow waves. Furthermore, in the group taking acetazolamide, a medication known to alleviate breathing disturbances, we controlled for one physiological parameter - breathing disturbances. Therefore, possibly another factor affected our measure of neuronal synchronization. In both OSA groups, at altitude the patients showed an increase in heart rate. We therefore hypothesized that an increase in sympathetic activation led to the observed changes, as these result are in accordance with our findings in the healthy group of participants examined in Zurich and at altitude.

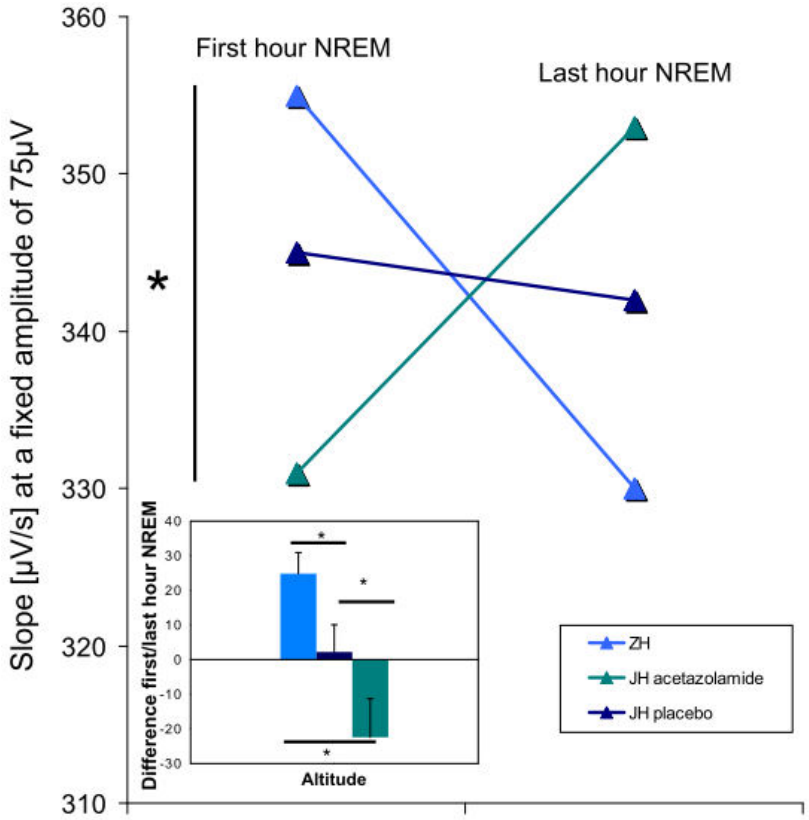

Figure 3: Overnight changes of the slope of slow waves from the first to the last hour of NREM sleep in a group of 30 patients with Obstructive Sleep Apnea (OSA) using a similar study design as in our healthy group. The differences in the first hour and in the last hour between the groups were compared separately by paired t-tests. Inset: Bars represent overnight differences of the slope from the first to the last hour of NREM sleep. Mean ± SEM of this difference at an amplitude of 75µV are shown for both altitudes, including 2 conditions at Jakobshorn (treated with placebo or acetazolamide). Differences between the groups were compared using paired t-test. * $p < 0.05$ indicate significant differences.

However, direct manipulations of sympathetic activity might prove a relationship between our measure of neuronal synchronization and sympathetic activity. The secretion of cortisol is also sympathetically regulated (van Stegeren et al., 2007) and correspondingly was shown to increase with a rise in altitude (Moncloa et al., 1967).

Furthermore, increased levels of cortisol impaired performance when cortisol was administered during sleep (Wagner and Born, 2008). Thus, an altitude dependent elevation of the cortisol level might have reduced the beneficial effects of sleep on memory performance. Whether this effect of cortisol on sleep dependent memory improvement is mediated via a change in the level of synchronization needs to be experimentally tested.

To our knowledge, this is the first study to demonstrate that a moderate increase in altitude reduces significantly the benefits of sleep on memory and furthermore that these alterations are related to slow waves. In other words, when travelling to moderate altitude, an important function of sleep in synaptic homeostasis seems to be impaired with negative consequences on sleep dependent memory performance. These findings may have important implications for a large number of people travelling to altitude, including further environments at which barometric pressures are similar to those at terrestrial altitudes, as for example aircraft-cabins (Muhm et al., 2009, Muhm et al., 2007). Future studies should take into account possible effects of acclimatization or subjects who already live at higher altitudes. Furthermore, studying patients with breathing disturbances as well as patients taking medication, known to alleviate breathing disturbances (e.g. acetazolamide), would be a next step to follow.

Taken together, our findings are consistent with a critical role of sleep for neuronal plasticity and memory while expanding this finding by showing that these beneficial effects can be dampened by natural environmental influences.

3.3. Final conclusions, limitations and outlook

In the first part of this thesis, I could highlight the importance of sleep research for understanding cortical plasticity during healthy development as well as in individuals affected by mental diseases. We demonstrated the potential of using hdEEG, hereby, spindle density and SWA topography as a tool to study early onset psychiatric disorders. Furthermore, SWA might represent a promising endophenotype which has the potential to identify subtle abnormalities not only in early-onset psychiatric diseases but already in adolescents at risk for developing psychiatric diseases as well as in healthy individuals. The perceptual disconnection from the environment during sleep might be a further advantage of hdEEG compared to other imaging techniques and particularly relevant for studies in children with psychiatric diseases.

Concerning the use of hdEEG in children and adolescents, there is a huge field of possibilities opening up, to study the relationship between cortical plasticity and

SWA in both health and disease. However, further studies are needed to establish SWA as a diagnostic tool that might be suitable for clinical use on the single subject level. As our approach proves to be promising, following individuals longitudinally would be a subsequent step to pursue. Furthermore, understanding healthy development and thereby disentangling long-term structural changes that may be irreversible from short-term dynamics would be a fundamental next aim to follow. For example, populations with extensive use of certain brain regions or with a special expertise are unique to study functional plasticity processes. Professional musicians show pronounced cortical reorganization in the somatosensory and auditory cortex (Elbert et al., 1995, Pantev et al., 1998), while the effect turned out to be larger the earlier the musicians started their education. This association between neuronal alterations and practice may offer a unique opportunity to study the interplay of short- and long-term plasticity processes. Investigating these processes in children and adolescents adds the dimension of brain maturation, which may offer a model to further disentangle the structural developmental differences from additional use-dependent and region-specific alterations. In this regard, a recent study indicated that processes of brain maturation favour experience-dependent plasticity. The data further confirmed that SWA is a highly sensitive tool to map maturational differences in experience-dependent plasticity while such plasticity processes seem to be highest during childhood (Wilhelm et al., 2014). Therefore, studying disturbed processes early on highlights the benefit of increased plasticity not only for early diagnosis but also for opportune treatment, thereby hopefully being able to steer maturation back to its normal course. A further advantage of studying early onset psychiatric diseases during such a period of increased plasticity is the opportunity to study disease development, thereby having the chance to eventually disentangle possible symptom progression on a continuum from no symptoms, to slight symptoms up to full blown symptoms while taking into account potential genetic vulnerabilities. This might help future research to identify what finally leads to the improper switch during maturation leading to disease onset.

Another aspect that should not be left unmentioned is the use of psychotropic medication and their subsequent effects. Due to our naturalistic study design the young patients continued their medication during the study period. Recently, a

study investigated untreated children and adolescents with attention deficit hyperactivity disorder (ADHD) as well as young patients treated with psychostimulants (Shaw et al., 2009). Compared to healthy controls, no slowing of overall cortical maturation was found in adolescents with ADHD taking medication. In contrast, faster regional decreases of cortical thickness were found in non-treated children and adolescents compared to both healthy controls as well as ADHD patients taking medication. However, no effects of medication on sleep EEG characteristics have been identified so far in youths with early onset psychiatric diseases. Adult patients with depression showed increased slow wave sleep after treatment with selective serotonin reuptake inhibitors (SSRIs) (Ehlers et al., 1996) while other studies showed an improvement of depressive symptoms after selective slow wave deprivation (Landsness et al., 2011). Concerning sleep spindles, healthy adult patients taking antipsychotics did not show any alterations in sleep spindles. Therefore, a reduction in sleep spindles in adult patients with schizophrenia can not be due to any side-effects of medication (Ferrarelli et al., 2010). However, how psychotropic medication affects sleep EEG characteristics together with symptom improvement need to be investigated in future studies. Ideally, upcoming studies should clearly separate groups of healthy subjects with and without medication from affected subjects with and without medication and if possible, follow them longitudinally over years, together with a change in symptom severity. As a further outlook also long-term effects of medication during a vulnerable developmental period is an issue that should be considered.

Next to the contribution of sleep in brain maturation (Kurth et al., 2012, Kurth et al., 2010b) there is increasing evidence that learning and memory benefit from sleep (Abel et al., 2013, Rasch and Born, 2013). So far, at least for the latter, several studies have done experimental manipulations by either boosting slow waves (Marshall et al., 2006) or depriving study participants from slow wave sleep (Landsness et al., 2009), leading to increases or decreases in memory performance after such manipulations. However, next to an improvement in learning and memory due to sleep compared to simply passage of time, it has been shown that slow waves are critical for the beneficial effects of sleep on memory (Huber et al., 2004, Landsness et al., 2009, Marshall et al., 2006, Ngo et al., 2013). However, how this happens exactly should be further investigated by

experimental and controlled manipulations by taking into account for example external influences. In the second part of this thesis, we manipulated environmental influences and indeed found that the beneficial effects of sleep on memory are susceptible to natural environmental influences, a moderate increase in altitude. This is an important finding since many people travel to more moderate altitudes for personal or professional purposes. Furthermore, hereby also other environments can be taken into account, at which barometric pressures are similar to those at terrestrial altitudes, as for example aircraft-cabins. Therefore, future studies should follow our findings and carefully study lower and higher altitudes, to find out, at which altitude such effects start to impair good functioning. Thus, individuals often confronted with higher altitude or who live or grew up in such environments should be carefully monitored. After understanding how these processes are altered in healthy individuals, older subjects or those with breathing or other disturbances should be taken into account.

Finally, I would like to address a few limitations of our studies. In the first part of the thesis, two major limitations should not be left unmentioned. First of all, the low sample size. Yet, during childhood and adolescence the incidence of early onset psychiatric diseases is rising but still quite low. Therefore, the number of participants to be considered was rather small during the whole study procedure. Furthermore, patients and parents pass through a hard time in their lives which makes a possible recruitment even more challenging. For a successful study progress, next to recruitment only, interest in study participation has to be gained and maintained, to make eventual longitudinal studies feasible. A second issue to mention are the comorbidities with other psychiatric disorders. However, this limitation is hard to be avoided as many psychiatric disorders share common symptoms, with some disorders preceding or following others. Thereby patients with comorbidities reflect a rather typical clinical sample. Future research in animal models could eventually separate between different disorders and symptoms. In the second part of the thesis, a major limitation was the absence of an adaptation night, which could have affected our findings. A further limitation was that we only investigated healthy male subjects at altitude. Future studies should also address gender differences, next to patients suffering from different disturbances.

In conclusion, this thesis yet again shows that sleep is more than just the passive counterpart of wakefulness. Specifically, sleep spindles and SWA may be a possible valuable mapping tool to display regions of interest in the context of disturbed processes. Furthermore, when undertaking external environmental manipulations, we could show that an important function of sleep in synaptic homeostasis seems to be impaired with negative consequences on sleep dependent memory performance.

4

References

Aasm Task Force. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The report of an American Academy of Sleep Medicine Task Force. *Sleep*, 1999, 22: 667-89.

Abel, T., Havekes, R., Saletin, J. M. and Walker, M. P. Sleep, plasticity and memory from molecules to whole-brain networks. *Curr Biol*, 2013, 23: R774-88.

Achermann, P. EEG Analysis applied to sleep. *Epileptologie*, 2009, 26: 28-33.

Achermann, P. and Borbely, A. A. Sleep homeostasis and models of sleep regulation. In Kryger, M. H., Roth, T., Dement, W. C. (eds). *Principles and Practice of Sleep Medicine. Elsevier Saunders, Philadelphia.*, 2011: pp. 405-17.

Aeschbach, D., Cutler, A. J. and Ronda, J. M. A role for non-rapid-eye-movement sleep homeostasis in perceptual learning. *J Neurosci*, 2008, 28: 2766-72.

Alfano, C. A., Ginsburg, G. S. and Kingery, J. N. Sleep-related problems among children and adolescents with anxiety disorders. *J Am Acad Child Adolesc Psychiatry*, 2007, 46: 224-32.

Amzica, F. and Steriade, M. Electrophysiological correlates of sleep delta waves. *Electroen Clin Neuro*, 1998, 107: 69-83.

Anderer, P., Klosch, G., Gruber, G., Trenker, E., Pascual-Marqui, R. D., Zeitlhofer, J., Barbanoj, M. J., Rappelsberger, P. and Saletu, B. Low-resolution brain electromagnetic tomography revealed simultaneously active frontal and parietal sleep spindle sources in the human cortex. *Neuroscience*, 2001, 103: 581-92.

Anders, T. F. Infant sleep, nighttime relationships, and attachment. *Psychiatry*, 1994, 57: 11-21.

Andreasen, N. C., Arndt, S., Swayze, V., 2nd, Cizadlo, T., Flaum, M., O'leary, D., Ehrhardt, J. C. and Yuh, W. T. Thalamic abnormalities in schizophrenia visualized through magnetic resonance image averaging. *Science*, 1994, 266: 294-8.

Anticevic, A., Cole, M. W., Repovs, G., Murray, J. D., Brumbaugh, M. S., Winkler, A. M., Savic, A., Krystal, J. H., Pearlson, G. D. and Glahn, D. C. Characterizing Thalamo-Cortical Disturbances in Schizophrenia and Bipolar Illness. *Cereb Cortex*, 2013

Armitage, R., Emslie, G. J., Hoffmann, R. F., Rintelmann, J. and Rush, A. J. Delta sleep EEG in depressed adolescent females and healthy controls. *J Affect Disorders*, 2001, 63: 139-48.

Armitage, R., Hoffmann, R., Trivedi, M. and Rush, A. J. Slow-wave activity in NREM sleep: sex and age effects in depressed outpatients and healthy controls. *Psychiat Res*, 2000, 95: 201-13.

Association, A. P. Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), Washington, DC: American Psychiatric Association. 1994

Association, A. P. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA; 2013., 2013

Ballageer, T., Malla, A., Manchanda, R., Takhar, J. and Haricharan, R. Is adolescent-onset first-episode psychosis different from adult onset? *J Am Acad Child Adolesc Psychiatry*, 2005, 44: 782-9.

Basner, M. and Dinges, D. F. Maximizing sensitivity of the psychomotor vigilance test (PVT) to sleep loss. *Sleep*, 2011, 34: 581-91.

Basso, M. R., Nasrallah, H. A., Olson, S. C. and Bornstein, R. A. Cognitive deficits distinguish patients with adolescent- and adult-onset schizophrenia. *Neuropsychiatry Neuropsychol Behav Neurol*, 1997, 10: 107-12.

Bat-Pitault, F., Da Fonseca, D., Cortese, S., Le Strat, Y., Kocher, L., Rey, M., Adrien, J., Deruelle, C. and Franco, P. The sleep macroarchitecture of children at risk for depression recruited in sleep centers. *Eur Psychiatry*, 2013, 28: 168-73.

Beaumont, M., Batejat, D., Coste, O., Van Beers, P., Colas, A., Clere, J. M. and Pierard, C. Effects of zolpidem and zaleplon on sleep, respiratory patterns and performance at a simulated altitude of 4,000 m. *Neuropsychobiology*, 2004, 49: 154-62.

Beaumont, M., Batejat, D., Pierard, C., Van Beers, P., Philippe, M., Leger, D., Savourey, G. and Jouanin, J. C. Zaleplon and zolpidem objectively alleviate sleep disturbances in mountaineers at a 3,613 meter altitude. *Sleep*, 2007, 30: 1527-33.

Beck, A. T. The evolution of the cognitive model of depression and its neurobiological correlates. *Am J Psychiatry*, 2008, 165: 969-77.

Benca, R. M., Obermeyer, W. H., Thisted, R. A. and Gillin, J. C. Sleep and Psychiatric-Disorders - a Metaanalysis. *Arch Gen Psychiat*, 1992, 49: 651-68.

Berk, M., Kapczinski, F., Andreazza, A. C.*, et al.* Pathways underlying neuroprogression in bipolar disorder: Focus on inflammation, oxidative stress and neurotrophic factors. *Neurosci Biobehav R*, 2011, 35: 804-17.

Biswas, P., Malhotra, S., Malhotra, A. and Gupta, N. Comparative study of neuropsychological correlates in schizophrenia with onset in childhood, adolescence and adulthood. *Eur Child Adolesc Psychiatry*, 2006, 15: 360-6.

Blake, H. and Gerard, R. W. Brain Potentials during Sleep. *Am J Physiol*, 1937, 119: 692-703.

Blakemore, S. J. and Choudhury, S. Development of the adolescent brain: implications for executive function and social cognition. *J Child Psychol Psychiatry*, 2006, 47: 296-312.

Bloch, K. E., Latshang, T. D., Turk, A. J.*, et al.* Nocturnal periodic breathing during acclimatization at very high altitude at Mount Muztagh Ata (7,546 m). *Am J Respir Crit Care Med*, 2010, 182: 562-8.

Bloch, K. E., Li, Y., Sackner, M. A. and Russi, E. W. Breathing pattern during sleep disruptive snoring. *Eur Respir J*, 1997, 10: 576-86.

Borbely, A. A. A two process model of sleep regulation. *Hum Neurobiol*, 1982, 1: 195-204.

Borbely, A. A. and Achermann, P. Sleep homeostasis and models of sleep regulation. In M.H. Kryger, T. Roth & W. C. Dement (Eds.), *Principles and Practice of Sleep Medicine* 2005: 405-17.

Borbely, A. A. and Wirz-Justice, A. Sleep, sleep deprivation and depression. A hypothesis derived from a model of sleep regulation. *Hum Neurobiol*, 1982, 1: 205-10.

Born, J., Rasch, B. and Gais, S. Sleep to remember. *Neuroscientist*, 2006, 12: 410-24.

Brown, L., Sherbenou, R., & Johnsen, S.K. Test of Nonverbal Intelligence. 1997, Third Edition (Vol. 3rd)

Buchmann, A., Ringli, M., Kurth, S., Schaerer, M., Geiger, A., Jenni, O. G. and Huber, R. EEG sleep slow-wave activity as a mirror of cortical maturation. *Cereb Cortex*, 2011, 21: 607-15.

Bunk, D., Eggers, C. and Klapal, M. Symptom dimensions in the course of childhood-onset schizophrenia. *Eur Child Adolesc Psychiatry*, 1999, 8 Suppl 1: I29-35.

Burgess, K. R., Johnson, P., Edwards, N. and Cooper, J. Acute mountain sickness is associated with sleep desaturation at high altitude. *Respirology*, 2004, 9: 485-92.

Campbell, I. G. and Feinberg, I. Longitudinal trajectories of non-rapid eye movement delta and theta EEG as indicators of adolescent brain maturation. *Proc Natl Acad Sci U S A*, 2009, 106: 5177-80.

Cannon, T. D., Rosso, I. M., Bearden, C. E., Sanchez, L. E. and Hadley, T. A prospective cohort study of neurodevelopmental processes in the genesis and epigenesis of schizophrenia. *Dev Psychopathol*, 1999, 11: 467-85.

Carskadon, M. A. and Acebo, C. A Self-Administered Rating-Scale for Pubertal Development. *J Adolescent Health*, 1993, 14: 190-95.

Carskadon, M. A. and Dement, W. C. Normal human sleep: An overview. In M.H. Kryger, T. Roth & W. C. Dement (Eds.),. In), *Principles and Practice of Sleep Medicine. WB Saunders Company, Philadelphia.*, 1994: 15-25.

Choe, N. N. The coordination of multi-joint reaching movements: A developmental profile Kingston., 2009

Chouinard, S., Poulin, J., Stip, E. and Godbout, R. Sleep in untreated patients with schizophrenia: a meta-analysis. *Schizophr Bull*, 2004, 30: 957-67.

Chugani, H. T. A critical period of brain development: studies of cerebral glucose utilization with PET. *Prev Med*, 1998, 27: 184-8.

Cirelli, C. and Tononi, G. Is sleep essential? *Plos Biol*, 2008, 6: 1605-11.

Clemmensen, L., Vernal, D. L. and Steinhausen, H. C. A systematic review of the long-term outcome of early onset schizophrenia. *BMC Psychiatry*, 2012, 12: 150.

Coble, P. A., Kupfer, D. J., Spiker, D. G., Neil, J. F. and Mcpartland, R. J. EEG sleep in primary depression. A longitudinal placebo study. *J Affect Disord*, 1979, 1: 131-8.

Cole, D. A., Martin, J. M., Peeke, L. G., Seroczynski, A. D. and Hoffman, K. Are cognitive errors of underestimation predictive or reflective of depressive symptoms in children: a longitudinal study. *J Abnorm Psychol*, 1998, 107: 481-96.

Colrain, I. M. and Baker, F. C. Changes in sleep as a function of adolescent development. *Neuropsychol Rev*, 2011a, 21: 5-21.

Colrain, I. M. and Baker, F. C. Sleep EEG, the clearest window through which to view adolescent brain development. *Sleep*, 2011b, 34: 1287-8.

Compas, B. E., Hinden, B. R. and Gerhardt, C. A. Adolescent development: pathways and processes of risk and resilience. *Annu Rev Psychol*, 1995, 46: 265-93.

Contreras, D. and Steriade, M. Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J Neurosci*, 1995, 15: 604-22.

Cooley, J. W. and Tukey, J. W. An Algorithm for Machine Calculation of Complex Fourier Series. *Math Comput*, 1965, 19: 297-&.

Cooney, R. E., Joormann, J., Eugene, F., Dennis, E. L. and Gotlib, I. H. Neural correlates of rumination in depression. *Cogn Affect Behav Neurosci*, 2010, 10: 470-8.

Cortese, S., Faraone, S. V., Konofal, E. and Lecendreux, M. Sleep in children with attention-deficit/hyperactivity disorder: meta-analysis of subjective and objective studies. *J Am Acad Child Adolesc Psychiatry*, 2009, 48: 894-908.

Coste, O., Van Beers, P. and Touitou, Y. Hypoxia-induced changes in recovery sleep, core body temperature, urinary 6-sulphatoxymelatonin and free cortisol after a simulated long-duration flight. *J Sleep Res*, 2009, 18: 454-65.

Crail-Melendez, D., Atriano-Mendieta, C., Carrillo-Meza, R. and Ramirez-Bermudez, J. Schizophrenia-like psychosis associated with right lacunar thalamic infarct. *Neurocase*, 2013, 19: 22-6.

Crunelli, V. and Hughes, S. W. The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat Neurosci*, 2010, 13: 9-17.

Cyranowski, J. M., Frank, E., Young, E. and Shear, M. K. Adolescent onset of the gender difference in lifetime rates of major depression: a theoretical model. *Arch Gen Psychiatry*, 2000, 57: 21-7.

Dahl, R. E., Ryan, N. D., Birmaher, B., Al-Shabbout, M., Williamson, D. E., Neidig, M., Nelson, B. and Puig-Antich, J. Electroencephalographic sleep measures in prepubertal depression. *Psychiatry Res*, 1991, 38: 201-14.

Dahl, R. E., Ryan, N. D., Matty, M. K., Birmaher, B., Al-Shabbout, M., Williamson, D. E. and Kupfer, D. J. Sleep onset abnormalities in depressed adolescents. *Biol Psychiatry*, 1996, 39: 400-10.

Dahl, R. E., Williamson, D. E., Bertocci, M. A., Stolz, M. V., Ryan, N. D. and Ehlers, C. L. Spectral analyses of sleep EEG in depressed offspring of fathers with or without a positive history of alcohol abuse or dependence: a pilot study. *Alcohol*, 2003, 30: 193-200.

Dale, A. M., Fischl, B. and Sereno, M. I. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage*, 1999, 9: 179-94.

Dang-Vu, T. T., Schabus, M., Desseilles, M.*, et al.* Spontaneous neural activity during human slow wave sleep. *P Natl Acad Sci USA*, 2008, 105: 15160-65.

Daseking, M., Petermann, U. and Petermann, F. Assessment of intelligence with the HAWIK-IV. *Kindh Entwickl*, 2007, 16: 250-59.

De Aquino Lemos, V., Antunes, H. K., Dos Santos, R. V., Lira, F. S., Tufik, S. and De Mello, M. T. High altitude exposure impairs sleep patterns, mood, and cognitive functions. *Psychophysiology*, 2012, 49: 1298-306.

De Gennaro, L. and Ferrara, M. Sleep spindles: an overview. *Sleep Med Rev*, 2003, 7: 423-40.

De Gennaro, L., Marzano, C., Fratello, F.*, et al.* The electroencephalographic fingerprint of sleep is genetically determined: a twin study. *Ann Neurol*, 2008, 64: 455-60.

Debas, K., Carrier, J., Orban, P.*, et al.* Brain plasticity related to the consolidation of motor sequence learning and motor adaptation. *Proc Natl Acad Sci U S A*, 2010, 107: 17839-44.

Defelipe, J. Types of neurons, synaptic connections and chemical characteristics of cells immunoreactive for calbindin-D28K, parvalbumin and calretinin in the neocortex. *J Chem Neuroanat*, 1997, 14: 1-19.

Delorme, A. and Makeig, S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Meth*, 2004, 134: 9-21.

Dempsey, J. A., Veasey, S. C., Morgan, B. J. and O'donnell, C. P. Pathophysiology of Sleep Apnea. *Physiol Rev*, 2010, 90: 47-112.

Diekelmann, S. and Born, J. The memory function of sleep. *Nat Rev Neurosci*, 2010, 11: 114-26.

Dijk, D. J. Slow-wave sleep deficiency and enhancement: implications for insomnia and its management. *World J Biol Psychiatry*, 2010, 11 Suppl 1: 22-8.

Dijk, D. J. and Beersma, D. G. Effects of SWS deprivation on subsequent EEG power density and spontaneous sleep duration. *Electroencephalogr Clin Neurophysiol*, 1989, 72: 312-20.

Dijk, D. J., Hayes, B. and Czeisler, C. A. Dynamics of electroencephalographic sleep spindles and slow wave activity in men: effect of sleep deprivation. *Brain Res*, 1993, 626: 190-9.

Drechsler, R., Brandeis, D., Foldenyi, M., Imhof, K. and Steinhausen, H. C. The course of neuropsychological functions in children with attention deficit hyperactivity disorder from late childhood to early adolescence. *J Child Psychol Psychiatry*, 2005, 46: 824-36.

Driver, H. S., Dijk, D. J., Werth, E., Biedermann, K. and Borbely, A. A. Sleep and the sleep electroencephalogram across the menstrual cycle in young healthy women. *J Clin Endocr Metab*, 1996, 81: 728-35.

Ducharme, S., Albaugh, M. D., Hudziak, J. J., Botteron, K. N., Nguyen, T. V., Truong, C., Evans, A. C. and Karama, S. Anxious/Depressed Symptoms are Linked to Right Ventromedial Prefrontal Cortical Thickness Maturation in Healthy Children and Young Adults. *Cereb Cortex*, 2013

Ehlers, C. L., Havstad, J. W. and Kupfer, D. J. Estimation of the time course of slow-wave sleep over the night in depressed patients: effects of clomipramine and clinical response. *Biol Psychiatry*, 1996, 39: 171-81.

Eichenberger, U., Weiss, E., Riemann, D., Oelz, O. and Bartsch, P. Nocturnal periodic breathing and the development of acute high altitude illness. *Am J Resp Crit Care*, 1996, 154: 1748-54.

Elbert, T., Pantev, C., Wienbruch, C., Rockstroh, B. and Taub, E. Increased Cortical Representation of the Fingers of the Left Hand in String Players. *Science*, 1995, 270: 305-07.

Emslie, G. J., Kennard, B. D., Mayes, T. L., Nakonezny, P. A., Zhu, L., Tao, R., Hughes, C. and Croarkin, P. Insomnia moderates outcome of serotonin-selective reuptake inhibitor treatment in depressed youth. *J Child Adolesc Psychopharmacol*, 2012, 22: 21-8.

Emslie, G. J., Rush, A. J., Weinberg, W. A., Rintelmann, J. W. and Roffwarg, H. P. Children with major depression show reduced rapid eye movement latencies. *Arch Gen Psychiatry*, 1990, 47: 119-24.

Esser, S. K., Hill, S. L. and Tononi, G. Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. *Sleep*, 2007, 30: 1617-30.

Fattinger, S., Jenni, O. G., Schmitt, B., Achermann, P. and Huber, R. Overnight changes in the slope of sleep slow waves during infancy. *Sleep*, 2014, 37: 245-53.

Feinberg, I. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *J Psychiatr Res*, 1982, 17: 319-34.

Feinberg, I., Braun, M., Koresko, R. L. and Gottlieb, F. Stage 4 sleep in schizophrenia. *Arch Gen Psychiatry*, 1969, 21: 262-6.

Feinberg, I. and Campbell, I. G. The onset of the adolescent delta power decline occurs after age 11 years: a comment on Tarokh and Carskadon. *Sleep*, 2010a, 33: 737; author reply 38.

Feinberg, I. and Campbell, I. G. Sleep EEG changes during adolescence: an index of a fundamental brain reorganization. *Brain Cogn*, 2010b, 72: 56-65.

Feinberg, I. and Campbell, I. G. Longitudinal sleep EEG trajectories indicate complex patterns of adolescent brain maturation. *Am J Physiol Regul Integr Comp Physiol*, 2013, 304: R296-303.

Feinberg, I., De Bie, E., Davis, N. M. and Campbell, I. G. Topographic differences in the adolescent maturation of the slow wave EEG during NREM sleep. *Sleep*, 2011, 34: 325-33.

Feinberg, I. and Floyd, T. C. Systematic trends across the night in human sleep cycles. *Psychophysiology*, 1979, 16: 283-91.

Feinberg, I., Higgins, L. M., Khaw, W. Y. and Campbell, I. G. The adolescent decline of NREM delta, an indicator of brain maturation, is linked to age and sex but not to pubertal stage. *Am J Physiol Regul Integr Comp Physiol*, 2006, 291: R1724-9.

Ferrarelli, F., Huber, R., Peterson, M. J., Massimini, M., Murphy, M., Riedner, B. A., Watson, A., Bria, P. and Tononi, G. Reduced sleep spindle activity in schizophrenia patients. *Am J Psychiatry*, 2007, 164: 483-92.

Ferrarelli, F., Peterson, M. J., Sarasso, S., Riedner, B. A., Murphy, M. J., Benca, R. M., Bria, P., Kalin, N. H. and Tononi, G. Thalamic dysfunction in schizophrenia suggested by whole-night deficits in slow and fast spindles. *Am J Psychiatry*, 2010, 167: 1339-48.

Ferrarelli, F. and Tononi, G. The thalamic reticular nucleus and schizophrenia. *Schizophr Bull*, 2011, 37: 306-15.

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J., Vos, T. and Whiteford, H. A. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med*, 2013, 10: e1001547.

Finelli, L. A., Achermann, P. and Borbely, A. A. Individual 'fingerprints' in human sleep EEG topography. *Neuropsychopharmacology*, 2001a, 25: S57-62.

Finelli, L. A., Borbely, A. A. and Achermann, P. Functional topography of the human nonREM sleep electroencephalogram. *European Journal of Neuroscience*, 2001b, 13: 2282-90.

Fischl, B., Sereno, M. I., Tootell, R. B. H. and Dale, A. M. High-resolution intersubject averaging and a coordinate system for the cortical surface. *Human Brain Mapping*, 1999, 8: 272-84.

Fogel, S. M., Nader, R., Cote, K. A. and Smith, C. T. Sleep spindles and learning potential. *Behav Neurosci*, 2007, 121: 1-10.

Forbes, E. E., Bertocci, M. A., Gregory, A. M., Ryan, N. D., Axelson, D. A., Birmaher, B. and Dahl, R. E. Objective sleep in pediatric anxiety disorders and major depressive disorder. *J Am Acad Child Adolesc Psychiatry*, 2008, 47: 148-55.

Franic, T., Kralj, Z., Marcinko, D., Knez, R. and Kardum, G. Suicidal ideations and sleep-related problems in early adolescence. *Early Interv Psychiatry*, 2013

Frank, M. G., Issa, N. P. and Stryker, M. P. Sleep enhances plasticity in the developing visual cortex. *Neuron*, 2001, 30: 275-87.

Frey, S., Birchler-Pedross, A., Hofstetter, M., Brunner, P., Gotz, T., Munch, M., Blatter, K., Knoblauch, V., Wirz-Justice, A. and Cajochen, C. Young women with major depression live on higher homeostatic sleep pressure than healthy controls. *Chronobiol Int*, 2012, 29: 278-94.

Galvan, A. Neural plasticity of development and learning. *Hum Brain Mapp*, 2010, 31: 879-90.

Geiger, A., Huber, R., Kurth, S., Ringli, M., Jenni, O. G. and Achermann, P. The sleep EEG as a marker of intellectual ability in school age children. *Sleep*, 2011, 34: 181-9.

Gogtay, N., Giedd, J. N., Lusk, L.*, et al.* Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci U S A*, 2004, 101: 8174-9.

Goldstein, T. R., Bridge, J. A. and Brent, D. A. Sleep disturbance preceding completed suicide in adolescents. *J Consult Clin Psychol*, 2008, 76: 84-91.

Green, H., Mcginnity A., Meltzer, H., Ford, T., Goodman, R. Mental health of children and young people in Great Britain. Basingstoke, UK: Palgrave Macmillan., 2005

Green, M. F., Nuechterlein, K. H., Gold, J. M.*, et al.* Approaching a consensus cognitive battery for clinical trials in schizophrenia: the NIMH-MATRICS conference to select cognitive domains and test criteria. *Biol Psychiatry*, 2004, 56: 301-7.

Greenough, W. T., Wallace, C. S., Alcantara, A. A., Anderson, B. J., Hawrylak, N., Sirevaag, A. M., Weiler, I. J. and Withers, G. S. Development of the brain: Experience affects the structure of neurons, glia, and blood vessels. In N. J. Anastasiow & S. Harll (Eds.). *At-risk infants: Interventions, families and research. New York: Paul H. Brookes*, 1993: pp. 173-85.

Gregory, A. M., Caspi, A., Eley, T. C., Moffitt, T. E., Oconnor, T. G. and Poulton, R. Prospective longitudinal associations between persistent sleep problems in childhood and anxiety and depression disorders in adulthood. *J Abnorm Child Psychol*, 2005, 33: 157-63.

Gregory, A. M. and O'connor, T. G. Sleep problems in childhood: a longitudinal study of developmental change and association with behavioural problems. *J Am Acad Child Adolesc Psychiatry*, 2002, 41: 964-71.

Gregory, A. M., Rijsdijk, F. V., Lau, J. Y., Dahl, R. E. and Eley, T. C. The direction of longitudinal associations between sleep problems and depression symptoms: a study of twins aged 8 and 10 years. *Sleep*, 2009, 32: 189-99.

Gruber, J., Miklowitz, D. J., Harvey, A. G., Frank, E., Kupfer, D., Thase, M. E., Sachs, G. S. and Ketter, T. A. Sleep matters: sleep functioning and course of illness in bipolar disorder. *J Affect Disord*, 2011, 134: 416-20.

Guillery, R. W., Feig, S. L. and Lozsadi, D. A. Paying attention to the thalamic reticular nucleus. *Trends Neurosci*, 1998, 21: 28-32.

Guillery, R. W. and Harting, J. K. Structure and connections of the thalamic reticular nucleus: Advancing views over half a century. *J Comp Neurol*, 2003, 463: 360-71.

Guller, Y., Ferrarelli, F., Shackman, A. J., Sarasso, S., Peterson, M. J., Langheim, F. J., Meyerand, M. E., Tononi, G. and Postle, B. R. Probing thalamic integrity in schizophrenia using concurrent transcranial magnetic stimulation and functional magnetic resonance imaging. *Arch Gen Psychiatry*, 2012, 69: 662-71.

Guo, Y., Nilsson, M. E., Heiligenstein, J., Wilson, M. G. and Emslie, G. An exploratory factor analysis of the children's depression rating scale-revised. *J Child Adolesc Psychopharmacol*, 2006, 16: 482-91.

Gur, R. E., Calkins, M. E., Gur, R. C., Horan, W. P., Nuechterlein, K. H., Seidman, L. J. and Stone, W. S. The Consortium on the Genetics of Schizophrenia: neurocognitive endophenotypes. *Schizophr Bull*, 2007, 33: 49-68.

Gustafsson, P., Holmstrom, E., Besjakov, J. and Karlsson, M. K. ADHD symptoms and maturity - a follow-up study in school children. *Acta Paediatr*, 2010, 99: 1536-9.

Guy, W. E. Clinical global impression scale. *In: ECDEU Assessment Manual for Psychopharmacology, revised. National Institute of Mental Health, Rockville, MD.*, 1976

Hackett, P. H. and Roach, R. C. High-altitude illness. *N Engl J Med*, 2001, 345: 107-14.

Hafner, H. and Nowotny, B. Epidemiology of early-onset schizophrenia. *Eur Arch Psychiatry Clin Neurosci*, 1995, 245: 80-92.

Hall, R. C. W. Global Assessment of Functioning - a Modified Scale. *Psychosomatics*, 1995, 36: 267-75.

Hansen, B. H., Skirbekk, B., Oerbeck, B., Wentzel-Larsen, T. and Kristensen, H. Associations Between Sleep Problems and Attentional and Behavioral Functioning in Children With Anxiety Disorders and ADHD. *Behav Sleep Med*, 2013

Hasler, G., Drevets, W. C., Manji, H. K. and Charney, D. S. Discovering endophenotypes for major depression. *Neuropsychopharmacology*, 2004, 29: 1765-81.

Hasler, G. and Northoff, G. Discovering imaging endophenotypes for major depression. *Mol Psychiatry*, 2011, 16: 604-19.

Hatzinger, M., Hemmeter, U. M., Brand, S., Ising, M. and Holsboer-Trachsler, E. Electroencephalographic sleep profiles in treatment course and long-term outcome of major depression: association with DEX/CRH-test response. *J Psychiatr Res*, 2004, 38: 453-65.

Hebb, D. O. The Organization of Behavior. *The Organization of Behavior. Lawrence Erlbaum Associates, New York.* , 1949

Heistad, D. D. and Abboud, F. M. Dickinson W. Richards Lecture: Circulatory adjustments to hypoxia. *Circulation*, 1980, 61: 463-70.

Hiatt, J. F., Floyd, T. C., Katz, P. H. and Feinberg, I. Further evidence of abnormal non-rapid-eye-movement sleep in schizophrenia. *Arch Gen Psychiatry*, 1985, 42: 797-802.

Hill, S. and Tononi, G. Modeling sleep and wakefulness in the thalamocortical system. *J Neurophysiol*, 2005, 93: 1671-98.

Hobson, J. A. and Pace-Schott, E. F. The cognitive neuroscience of sleep: Neuronal systems, consciousness and learning. *Nat Rev Neurosci*, 2002, 3: 679-93.

Hoffman, R. E. and Mcglashan, T. H. Neurodynamics and Schizophrenia Research - Editors Introduction. *Schizophrenia Bull*, 1993, 19: 15-19.

Hollingshead, A. B. Four factor index of social status. *Unpublished manuscript. Yale University, New Haven, CT.*, 1975

Hollis, C. Adult outcomes of child- and adolescent-onset schizophrenia: diagnostic stability and predictive validity. *Am J Psychiatry*, 2000, 157: 1652-9.

Hollis, C. Developmental precursors of child- and adolescent-onset schizophrenia and affective psychoses: diagnostic specificity and continuity with symptom dimensions. *Br J Psychiatry*, 2003, 182: 37-44.

Huber, R. and Born, J. Sleep, synaptic connectivity, and hippocampal memory during early development. *Trends Cogn Sci*, 2014, 18: 141-52.

Huber, R., Esser, S. K., Ferrarelli, F., Massimini, M., Peterson, M. J. and Tononi, G. TMS-induced cortical potentiation during wakefulness locally increases slow wave activity during sleep. *PLoS One*, 2007, 2: e276.

Huber, R., Ghilardi, M. F., Massimini, M., Ferrarelli, F., Riedner, B. A., Peterson, M. J. and Tononi, G. Arm immobilization causes cortical plastic changes and

locally decreases sleep slow wave activity. *Nature Neuroscience*, 2006, 9: 1169-76.

Huber, R., Ghilardi, M. F., Massimini, M. and Tononi, G. Local sleep and learning. *Nature*, 2004, 430: 78-81.

Huber, R., Graf, T., Cote, K. A., Wittmann, L., Gallmann, E., Matter, D., Schuderer, J., Kuster, N., Borbely, A. A. and Achermann, P. Exposure to pulsed high-frequency electromagnetic field during waking affects human sleep EEG. *Neuroreport*, 2000, 11: 3321-5.

Huddleston B., Ataman E., De Salvo P. and Al., E. Towards a GIS-based analysis of mountain environments and populations. Rome, Italy: Food and Agricultural Organization of the United Nations., 2003

Huguenard, J. R. Low-threshold calcium currents in central nervous system neurons. *Annu Rev Physiol*, 1996, 58: 329-48.

Huttenlocher, P. R. Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Res*, 1979, 163: 195-205.

Huttenlocher, P. R. Morphometric study of human cerebral cortex development. *Neuropsychologia*, 1990, 28: 517-27.

Huttenlocher, P. R. and Dabholkar, A. S. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol*, 1997, 387: 167-78.

Hyman, S., Chisholm, D., Kessler, R., Patel, V. and Whiteford, H. Mental Disorders. 2006

Iber, C., Ancoli-Israle, S., Chesson, A.L. & Wuan, S.F. The AASM Manual for the scoring of sleep and associated events. *American Academy of Sleep Medicine, Westchester, Illinois, USA.*, 2007

Jackson, A., Cavanagh, J. and Scott, J. A systematic review of manic and depressive prodromes. *J Affect Disord*, 2003, 74: 209-17.

Jacobsen, L. K. and Rapoport, J. L. Research update: childhood-onset schizophrenia: implications of clinical and neurobiological research. *J Child Psychol Psychiatry*, 1998, 39: 101-13.

Jansen, P. W., Saridjan, N. S., Hofman, A., Jaddoe, V. W., Verhulst, F. C. and Tiemeier, H. Does disturbed sleeping precede symptoms of anxiety or depression in toddlers? The generation R study. *Psychosom Med*, 2011, 73: 242-9.

Jenkins, J. G. and Dallenbach, K. M. Obliviscence during sleep and waking. *Am J Psychol*, 1924, 35: 605-12.

Jenkins, J. H. and Schumacher, J. G. Family burden of schizophrenia and depressive illness. Specifying the effects of ethnicity, gender and social ecology. *Br J Psychiatry*, 1999, 174: 31-8.

Jenni, O. G., Van Reen, E. and Carskadon, M. A. Regional differences of the sleep electroencephalogram in adolescents. *J Sleep Res*, 2005, 14: 141-7.

Johnson, M. H. Functional brain development in humans. *Nat Rev Neurosci*, 2001, 2: 475-83.

Johnson, P. L., Edwards, N., Burgess, K. R. and Sullivan, C. E. Sleep architecture changes during a trek from 1400 to 5000 m in the Nepal Himalaya. *J Sleep Res*, 2010, 19: 148-56.

Jones, E. G. The Thalamus. *Cambridge University Press, Cambridge, UK*, 2007

Juniper, M., Hack, M. A., George, C. F., Davies, R. J. and Stradling, J. R. Steering simulation performance in patients with obstructive sleep apnoea and matched control subjects. *Eur Respir J*, 2000, 15: 590-5.

Jus, K., Kiljan, A., Wilczak, H., Kubacki, A., Rzepecki, J. and Jus, A. [Polygraphic study of night sleep in schizophrenia before and during treatment with phenothiazine derivatives]. *Ann Med Psychol (Paris)*, 1968, 126: 713-25.

Kaida, K., Takahashi, M., Akerstedt, T., Nakata, A., Otsuka, Y., Haratani, T. and Fukasawa, K. Validation of the Karolinska sleepiness scale against performance and EEG variables. *Clin Neurophysiol*, 2006, 117: 1574-81.

Kandel, A. and Buzsaki, G. Cellular-synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of the rat. *J Neurosci*, 1997, 17: 6783-97.

Kattler, H., Dijk, D. J. and Borbely, A. A. Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. *J Sleep Res*, 1994, 3: 159-64.

Kay, S. R., Fiszbein, A. and Opler, L. A. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull*, 1987, 13: 261-76.

Keller, F., Grieb, J., Ernst, M., Sprober, N., Fegert, J. M. and Kolch, M. [Children's Depression Rating Scale-Revised (CDRS-R): development of a German version and psychometric properties in a clinical sample]. *Z Kinder Jugendpsychiatr Psychother*, 2011, 39: 179-85.

Kelly, K. R., Williamson, D. L., Fealy, C. E., Kriz, D. A., Krishnan, R. K., Huang, H., Ahn, J., Loomis, J. L. and Kirwan, J. P. Acute altitude-induced hypoxia suppresses plasma glucose and leptin in healthy humans. *Metabolism*, 2010, 59: 200-5.

Kerr, A. L., Cheng, S. Y. and Jones, T. A. Experience-dependent neural plasticity in the adult damaged brain. *J Commun Disord*, 2011, 44: 538-48.

Kessler, R. C., Avenevoli, S., Costello, E. J.*, et al.* Prevalence, persistence, and sociodemographic correlates of DSM-IV disorders in the National Comorbidity Survey Replication Adolescent Supplement. *Arch Gen Psychiatry*, 2012, 69: 372-80.

Kessler, R. C., Avenevoli, S. and Ries Merikangas, K. Mood disorders in children and adolescents: an epidemiologic perspective. *Biol Psychiatry*, 2001, 49: 1002-14.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K. R., Rush, A. J., Walters, E. E. and Wang, P. S. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *JAMA*, 2003, 289: 3095-105.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R. and Walters, E. E. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*, 2005, 62: 593-602.

Khoo, M. C., Kronauer, R. E., Strohl, K. P. and Slutsky, A. S. Factors inducing periodic breathing in humans: a general model. *J Appl Physiol Respir Environ Exerc Physiol*, 1982, 53: 644-59.

Kinsbourne, M. Minimal brain dysfunction as a neurodevelopmental lag. *Ann N Y Acad Sci*, 1973, 205: 268-73.

Kirkwood, B. R. and Sterne, J. A. C. Essential medical statisitcs. 2 ed. Oxford: Blackwell Publishers., 2003

Klein, D. C., Moore, R. Y. and Reppert, S. M. Suprachiasmatic Nucleus. The Mind's Clock. *Oxford University Press, New York.*, 1991

Kohler, M., Kriemler, S., Wilhelm, E. M., Brunner-Larocca, H., Zehnder, M. and Bloch, K. E. Children at high altitude have less nocturnal periodic breathing than adults. *Eur Respir J*, 2008, 32: 189-97.

Konofal, E., Lecendreux, M. and Cortese, S. Sleep and ADHD. *Sleep Med*, 2010, 11: 652-8.

Krakauer, J. W., Ghilardi, M. F. and Ghez, C. Independent learning of internal models for kinematic and dynamic control of reaching. *Nat Neurosci*, 1999, 2: 1026-31.

Krakauer, J. W. and Shadmehr, R. Consolidation of motor memory. *Trends Neurosci*, 2006, 29: 58-64.

Kurth, S., Jenni, O. G., Riedner, B. A., Tononi, G., Carskadon, M. A. and Huber, R. Characteristics of sleep slow waves in children and adolescents. *Sleep*, 2010a, 33: 475-80.

Kurth, S., Ringli, M., Geiger, A., Lebourgeois, M., Jenni, O. G. and Huber, R. Mapping of cortical activity in the first two decades of life: a high-density sleep electroencephalogram study. *J Neurosci*, 2010b, 30: 13211-9.

Kurth, S., Ringli, M., Lebourgeois, M. K., Geiger, A., Buchmann, A., Jenni, O. G. and Huber, R. Mapping the electrophysiological marker of sleep depth reveals skill maturation in children and adolescents. *Neuroimage*, 2012, 63: 959-65.

Landolt, H. P. Genetic determination of sleep EEG profiles in healthy humans. *Prog Brain Res*, 2011, 193: 51-61.

Landsness, E. C., Crupi, D., Hulse, B. K.*, et al.* Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. *Sleep*, 2009, 32: 1273-84.

Landsness, E. C., Goldstein, M. R., Peterson, M. J., Tononi, G. and Benca, R. M. Antidepressant effects of selective slow wave sleep deprivation in major depression: a high-density EEG investigation. *J Psychiatr Res*, 2011, 45: 1019-26.

Latshang, T. D., Lo Cascio, C. M., Stowhas, A. C., Grimm, M., Stadelmann, K., Tesler, N., Achermann, P., Huber, R., Kohler, M. and Bloch, K. E. Are Nocturnal Breathing, Sleep, and Cognitive Performance Impaired at Moderate Altitude (1,630-2,590 m)? *Sleep*, 2013, 36: 1969-76.

Latshang, T. D., Nussbaumer-Ochsner, Y., Henn, R. M., Ulrich, S., Lo Cascio, C. M., Ledergerber, B., Kohler, M. and Bloch, K. E. Effect of acetazolamide and autoCPAP therapy on breathing disturbances among patients with

obstructive sleep apnea syndrome who travel to altitude: a randomized controlled trial. *JAMA*, 2012, 308: 2390-8.

Lenroot, R. K. and Giedd, J. N. Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neurosci Biobehav Rev*, 2006, 30: 718-29.

Leopold, K., Ritter, P., Correll, C. U., Marx, C., Ozgurdal, S., Juckel, G., Bauer, M. and Pfennig, A. Risk constellations prior to the development of bipolar disorders: rationale of a new risk assessment tool. *J Affect Disord*, 2012, 136: 1000-10.

Lieb, S. Principles of Adult Learning. *Retrieved April, 17th, 2006, from www.hcc.hawaii.edu/intranet/committees/FacDevCom/guidebk/teachtip/adults-2.htm*, 1999

Light, G. A. and Braff, D. L. Human and animal studies of schizophrenia-related gating deficits. *Curr Psychiatry Rep*, 1999, 1: 31-40.

Liu, X., Buysse, D. J., Gentzler, A. L., Kiss, E., Mayer, L., Kapornai, K., Vetro, A. and Kovacs, M. Insomnia and hypersomnia associated with depressive phenomenology and comorbidity in childhood depression. *Sleep*, 2007, 30: 83-90.

Lopez, J., Hoffmann, R., Emslie, G. and Armitage, R. Sex differences in slow-wave electroencephalographic activity (SWA) in adolescent depression. *Mental Illness*, 2012, 4: 15-20.

Lubenov, E. V. and Siapas, A. G. Decoupling through synchrony in neuronal circuits with propagation delays. *Neuron*, 2008, 58: 118-31.

Luks, A. M., Van Melick, H., Batarse, R. R., Powell, F. L., Grant, I. and West, J. B. Room oxygen enrichment improves sleep and subsequent day-time performance at high altitude. *Resp Physiol*, 1998, 113: 247-58.

Lunsford-Avery, J. R., Judd, C. M., Axelson, D. A. and Miklowitz, D. J. Sleep impairment, mood symptoms, and psychosocial functioning in adolescent bipolar disorder. *Psychiatry Res*, 2012, 200: 265-71.

Lustenberger, C. and Huber, R. High density electroencephalography in sleep research: potential, problems, future perspective. *Front Neurol*, 2012, 3: 77.

Lustenberger, C., O'gorman, R.L., Tüshaus, L., Wehrle, F., Achermann, P. & Huber R. Sleep spindles predict schizotypal personality traits and thalamic

Glutamine/Glutamate in healthy subjects. *Schizophr Bull*, 2014, epub ahead of print

Maarsingh, E. J., Van Eykern, L. A., Sprikkelman, A. B., Hoekstra, M. O. and Van Aalderen, W. M. Respiratory muscle activity measured with a noninvasive EMG technique: technical aspects and reproducibility. *J Appl Physiol (1985)*, 2000, 88: 1955-61.

Maatta, S., Landsness, E., Sarasso, S., Ferrarelli, F., Ferreri, F., Ghilardi, M. F. and Tononi, G. The effects of morning training on night sleep: a behavioral and EEG study. *Brain Res Bull*, 2010, 82: 118-23.

Mackintosh, J. H., Thomas, D. J., Olive, J. E., Chesner, I. M. and Knight, R. J. E. The Effect of Altitude on Tests of Reaction-Time and Alertness. *Aviat Space Envir Md*, 1988, 59: 246-48.

Maggiorini, M., Buhler, B., Walter, M. and Oelz, O. Prevalence of acute mountain sickness in the Swiss Alps. *BMJ*, 1990, 301: 853-5.

Maggiorini, M., Muller, A., Hofstetter, D., Bartsch, P. and Oelz, O. Assessment of acute mountain sickness by different score protocols in the Swiss Alps. *Aviat Space Environ Med*, 1998, 69: 1186-92.

Malenka, R. C. and Bear, M. F. LTP and LTD: An embarrassment of riches. *Neuron*, 2004, 44: 5-21.

Malenka, R. C. and Nicoll, R. A. Long-term potentiation--a decade of progress? *Science*, 1999, 285: 1870-4.

Manoach, D. S., Demanuele, C., Wamsley, E. J., Vangel, M., Montrose, D. M., Miewald, J., Kupfer, D., Buysse, D., Stickgold, R. and Keshavan, M. S. Sleep spindle deficits in antipsychotic-naive early course schizophrenia and in non-psychotic first-degree relatives. *Front Hum Neurosci*, 2014, 8: 762.

March, J. S., Silva, S., Petrycki, S.*, et al.* The Treatment for Adolescents With Depression Study (TADS): long-term effectiveness and safety outcomes. *Arch Gen Psychiatry*, 2007, 64: 1132-43.

Marshall, L., Helgadottir, H., Molle, M. and Born, J. Boosting slow oscillations during sleep potentiates memory. *Nature*, 2006, 444: 610-13.

Mason, T. B., Teoh, L., Calabro, K., Traylor, J., Karamessinis, L., Schultz, B., Samuel, J., Gallagher, P. R. and Marcus, C. L. Rapid eye movement latency in children and adolescents. *Pediatr Neurol*, 2008, 39: 162-9.

McAlonan, G. M., Daly, E., Kumari, V.*, et al.* Brain anatomy and sensorimotor gating in Asperger's syndrome. *Brain*, 2002, 125: 1594-606.

Mcgorry, P. D., Purcell, R., Goldstone, S. and Amminger, G. P. Age of onset and timing of treatment for mental and substance use disorders: implications for preventive intervention strategies and models of care. *Curr Opin Psychiatr*, 2011, 24: 301-06.

Merikangas, K. R., He, J. P., Burstein, M., Swanson, S. A., Avenevoli, S., Cui, L., Benjet, C., Georgiades, K. and Swendsen, J. Lifetime prevalence of mental disorders in U.S. adolescents: results from the National Comorbidity Survey Replication--Adolescent Supplement (NCS-A). *J Am Acad Child Adolesc Psychiatry*, 2010, 49: 980-9.

Miller, E. M. Intelligence and Brain Myelination - a Hypothesis. *Pers Indiv Differ*, 1994, 17: 803-32.

Miyamoto, H., Katagiri, H. and Hensch, T. Experience-dependent slow-wave sleep development. *Nature Neuroscience*, 2003, 6: 553-4.

Mizuno, K., Asano, K. and Okudaira, N. Sleep and Respiration under Acute Hypobaric Hypoxia. *Jpn J Physiol*, 1993, 43: 161-75.

Modell, S., Ising, M., Holsboer, F. and Lauer, C. J. The Munich Vulnerability Study on Affective Disorders: stability of polysomnographic findings over time. *Biol Psychiatry*, 2002, 52: 430-7.

Modell, S., Ising, M., Holsboer, F. and Lauer, C. J. The Munich vulnerability study on affective disorders: premorbid polysomnographic profile of affected high-risk probands. *Biol Psychiatry*, 2005, 58: 694-9.

Moncloa, F., Velasco, I. and Beteta, L. Plasma Cortisol Concentration and Disappearance Rate of 4-14c-Cortisol in Newcomers to High Altitude. *Acta Endocrinol-Cop*, 1967, S 56: 36-&.

Muhm, J. M., Rock, P. B., Mcmullin, D. L., Jones, S. P., Lu, I. L., Eilers, K. D., Space, D. R. and Mcmullen, A. Effect of aircraft-cabin altitude on passenger discomfort. *N Engl J Med*, 2007, 357: 18-27.

Muhm, J. M., Signal, T. L., Rock, P. B., Jones, S. P., O'keeffe, K. M., Weaver, M. R., Zhu, S., Gander, P. H. and Belenky, G. Sleep at simulated 2438 m: effects on oxygenation, sleep quality, and postsleep performance. *Aviat Space Environ Med*, 2009, 80: 691-7.

Murphy, F. C., Sahakian, B. J., Rubinsztein, J. S., Michael, A., Rogers, R. D., Robbins, T. W. and Paykel, E. S. Emotional bias and inhibitory control processes in mania and depression. *Psychol Med*, 1999, 29: 1307-21.

Murphy, M., Riedner, B. A., Huber, R., Massimini, M., Ferrarelli, F. and Tononi, G. Source modeling sleep slow waves. *Proc Natl Acad Sci U S A*, 2009, 106: 1608-13.

Nere, A., Hashmi, A., Cirelli, C. and Tononi, G. Sleep-dependent synaptic down-selection (I): modeling the benefits of sleep on memory consolidation and integration. *Front Neurol*, 2013, 4: 143.

Ngo, H. V., Martinetz, T., Born, J. and Molle, M. Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron*, 2013, 78: 545-53.

Nichols, T. E. and Holmes, A. P. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp*, 2002, 15: 1-25.

Nicolas, A., Petit, D., Rompre, S. and Montplaisir, J. Sleep spindle characteristics in healthy subjects of different age groups. *Clin Neurophysiol*, 2001, 112: 521-7.

Nock, M. K., Hwang, I., Sampson, N. A. and Kessler, R. C. Mental disorders, comorbidity and suicidal behavior: Results from the National Comorbidity Survey Replication. *Mol Psychiatr*, 2010, 15: 868-76.

Nussbaumer-Ochsner, Y. and Bloch, K. E. Sleep. In: S. E. R. and B. P. (Eds), *High Altitude. Human Adaptation to Hypoxia*. Springer, New York

Heidelberg, 2014: 325-40.

Nussbaumer-Ochsner, Y., Latshang, T. D., Ulrich, S., Kohler, M., Thurnheer, R. and Bloch, K. E. Patients with obstructive sleep apnea syndrome benefit from acetazolamide during an altitude sojourn: a randomized, placebo-controlled, double-blind trial. *Chest*, 2012a, 141: 131-8.

Nussbaumer-Ochsner, Y., Schuepfer, N., Ulrich, S. and Bloch, K. E. Exacerbation of sleep apnoea by frequent central events in patients with the obstructive sleep apnoea syndrome at altitude: a randomised trial. *Thorax*, 2010, 65: 429-35.

Nussbaumer-Ochsner, Y., Schuepfer, N., Ursprung, J., Siebenmann, C., Maggiorini, M. and Bloch, K. E. Sleep and breathing in high altitude pulmonary edema susceptible subjects at 4,559 meters. *Sleep*, 2012b, 35: 1413-21.

Nussbaumer-Ochsner, Y., Ursprung, J., Siebenmann, C., Maggiorini, M. and Bloch, K. E. Effect of Short-Term Acclimatization to High Altitude on Sleep and Nocturnal Breathing. *Sleep*, 2012c, 35: 419-23.

Organization, W. T. UNWTO Tourism Highlights 2012 Edition. Madrid, Spain. 2012

Palagini, L., Baglioni, C., Ciapparelli, A., Gemignani, A. and Riemann, D. REM sleep dysregulation in depression: State of the art. *Sleep Med Rev*, 2013

Pantev, C., Oostenveld, R., Engelien, A., Ross, B., Roberts, L. E. and Hoke, M. Increased auditory cortical representation in musicians. *Nature*, 1998, 392: 811-14.

Patton, G. C. and Viner, R. Pubertal transitions in health. *Lancet*, 2007, 369: 1130-9.

Paus, T. Mapping brain maturation and cognitive development during adolescence. *Trends Cogn Sci*, 2005, 9: 60-8.

Paus, T., Keshavan, M. and Giedd, J. N. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci*, 2008, 9: 947-57.

Perlis, R. H., Miyahara, S., Marangell, L. B., Wisniewski, S. R., Ostacher, M., Delbello, M. P., Bowden, C. L., Sachs, G. S. and Nierenberg, A. A. Long-term implications of early onset in bipolar disorder: data from the first 1000 participants in the systematic treatment enhancement program for bipolar disorder (STEP-BD). *Biol Psychiatry*, 2004, 55: 875-81.

Poulin, J., Daoust, A. M., Forest, G., Stip, E. and Godbout, R. Sleep architecture and its clinical correlates in first episode and neuroleptic-naive patients with schizophrenia. *Schizophr Res*, 2003, 62: 147-53.

Poznanski, E. O., Mokros, H.B. Children's Depression Rating Scale, Revised (CDRS-R): Manual. *Los Angeles: Western Psychological Services*, 1996

Programme, U. N. E. Mountain tourism. United Nations Environmental Programme, Division of Technology, Industry and Economics., 2009

Rao, U., Dahl, R. E., Ryan, N. D., Birmaher, B., Williamson, D. E., Rao, R. and Kaufman, J. Heterogeneity in EEG sleep findings in adolescent depression: unipolar versus bipolar clinical course. *J Affect Disord*, 2002, 70: 273-80.

Rasch, B. and Born, J. About sleep's role in memory. *Physiol Rev*, 2013, 93: 681-766.

Reato, D., Gasca, F., Datta, A., Bikson, M., Marshall, L. and Parra, L. C. Transcranial electrical stimulation accelerates human sleep homeostasis. *PLoS Comput Biol*, 2013, 9: e1002898.

Rechtschaffen, A., Kales A. A manual of standardized terminology, techniques and scoring system for sleep stagesof human subjects. *Washington, DC: US Public Health Service, US Government Printing Office*, 1968

Regard, M., Landis, T., Casey, J., Maggiorini, M., Bartsch, P. and Oelz, O. Cognitive Changes at High-Altitude in Healthy Climbers and in Climbers Developing Acute Mountain-Sickness. *Aviat Space Envir Md*, 1991, 62: 291-95.

Regel, S. J., Tinguely, G., Schuderer, J., Adam, M., Kuster, N., Landolt, H. P. and Achermann, P. Pulsed radio-frequency electromagnetic fields: dose-dependent effects on sleep, the sleep EEG and cognitive performance. *J Sleep Res*, 2007, 16: 253-8.

Reid, S. N. and Daw, N. W. Dark-rearing changes dendritic microtubule-associated protein 2 (MAP2) but not subplate neurons in cat visual cortex. *J Comp Neurol*, 1995, 359: 38-47.

Reite, M., Jackson, D., Cahoon, R. L. and Weil, J. V. Sleep physiology at high altitude. *Electroencephalogr Clin Neurophysiol*, 1975, 38: 463-71.

Reynolds, C. F. and Kupfer, D. J. Sleep research in affective illness: state of the art circa 1987. *Sleep*, 1987, 10: 199-215.

Reynolds, S., Carrey, N., Jaworska, N., Langevin, L. M., Yang, X. R. and Macmaster, F. P. Cortical thickness in youth with major depressive disorder. *BMC Psychiatry*, 2014, 14: 83.

Rice, F., Harold, G. and Thapar, A. The genetic aetiology of childhood depression: a review. *J Child Psychol Psychiatry*, 2002, 43: 65-79.

Riedner, B. A., Vyazovskiy, V. V., Huber, R., Massimini, M., Esser, S., Murphy, M. and Tononi, G. Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. *Sleep*, 2007, 30: 1643-57.

Riemann, D. and Voderholzer, U. Primary insomnia: a risk factor to develop depression? *J Affect Disord*, 2003, 76: 255-9.

Ringli, M. and Huber, R. Developmental aspects of sleep slow waves: Linking sleep, brain maturation and behavior. *Slow Brain Oscillations of Sleep, Resting State and Vigilance*, 2011, 193: 63-82.

Ringli, M., Souissi, S., Kurth, S., Brandeis, D., Jenni, O. G. and Huber, R. Topography of sleep slow wave activity in children with attention-deficit/hyperactivity disorder. *Cortex*, 2013, 49: 340-7.

Roenneberg, T., Kuehnle, T., Pramstaller, P. P., Ricken, J., Havel, M., Guth, A. and Merrow, M. A marker for the end of adolescence. *Curr Biol*, 2004, 14: R1038-9.

Roffwarg, H. P., Muzio, J. N. and Dement, W. C. Ontogenetic development of the human sleep-dream cycle. *Science*, 1966, 152: 604-19.

Rowell, L. B., Saltin, B., Kiens, B. and Christensen, N. J. Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia? *Am J Physiol*, 1986, 251: H1038-44.

Rutter, M., Kim-Cohen, J. and Maughan, B. Continuities and discontinuities in psychopathology between childhood and adult life. *J Child Psychol Psychiatry*, 2006, 47: 276-95.

Sackner, M. A., Watson, H., Belsito, A. S., Feinerman, D., Suarez, M., Gonzalez, G., Bizousky, F. and Krieger, B. Calibration of respiratory inductive plethysmograph during natural breathing. *J Appl Physiol (1985)*, 1989, 66: 410-20.

Sadeh, A., Pergamin, L. and Bar-Haim, Y. Sleep in children with attention-deficit hyperactivity disorder: A meta-analysis of polysomnographic studies. *Sleep Medicine Reviews*, 2006, 10: 381-98.

Salvaggio, A., Insalaco, G., Marrone, O., Romano, S., Braghiroli, A., Lanfranchi, P., Patruno, V., Donner, C. F. and Bonsignore, G. Effects of high-altitude periodic breathing on sleep and arterial oxyhaemoglobin saturation. *Eur Respir J*, 1998, 12: 408-13.

Samartzis, L., Dima, D., Fusar-Poli, P. and Kyriakopoulos, M. White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. *J Neuroimaging*, 2014, 24: 101-10.

Sampson, J. B., Cymerman, A., Burse, R. L., Maher, J. T. and Rock, P. B. Procedures for the measurement of acute mountain sickness. *Aviat Space Environ Med*, 1983, 54: 1063-73.

Savitz, J. B. and Drevets, W. C. Imaging phenotypes of major depressive disorder: genetic correlates. *Neuroscience*, 2009, 164: 300-30.

Schimmelmann, B. G., Conus, P., Cotton, S., Mcgorry, P. D. and Lambert, M. Pre-treatment, baseline, and outcome differences between early-onset and adult-onset psychosis in an epidemiological cohort of 636 first-episode patients. *Schizophr Res*, 2007, 95: 1-8.

Schimmelmann, B. G., Conus, P., Edwards, J., Mcgorry, P. D. and Lambert, M. Diagnostic stability 18 months after treatment initiation for first-episode psychosis. *J Clin Psychiatry*, 2005, 66: 1239-46.

Schneiberg, S., Sveistrup, H., Mcfadyen, B., Mckinley, P. and Levin, M. F. The development of coordination for reach-to-grasp movements in children. *Exp Brain Res*, 2002, 146: 142-54.

Schwartz, P. J., Rosenthal, N. E. and Wehr, T. A. Band-specific electroencephalogram and brain cooling abnormalities during NREM sleep in patients with winter depression. *Biol Psychiatry*, 2001, 50: 627-32.

Scott, N., Blair, P. S., Emond, A. M., Fleming, P. J., Humphreys, J. S., Henderson, J. and Gringras, P. Sleep patterns in children with ADHD: a population-based cohort study from birth to 11years. *Journal of Sleep Research*, 2013, 22: 121-28.

Sejnowski, T. J. and Destexhe, A. Why do we sleep? *Brain Res*, 2000, 886: 208-23.

Selvamurthy, W., Saxena, R. K., Krishnamurthy, N. and Nayar, H. S. Autonomic Responses of High-Altitude Natives during Sojourn at Plains and on Return to Altitude. *Aviat Space Envir Md*, 1981, 52: 346-49.

Shaw, P., Eckstrand, K., Sharp, W., Blumenthal, J., Lerch, J. P., Greenstein, D., Clasen, L., Evans, A., Giedd, J. and Rapoport, J. L. Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proc Natl Acad Sci U S A*, 2007, 104: 19649-54.

Shaw, P., Kabani, N. J., Lerch, J. P.*, et al.* Neurodevelopmental trajectories of the human cerebral cortex. *J Neurosci*, 2008, 28: 3586-94.

Shaw, P., Sharp, W. S., Morrison, M., Eckstrand, K., Greenstein, D. K., Clasen, L. S., Evans, A. C. and Rapoport, J. L. Psychostimulant treatment and the developing cortex in attention deficit hyperactivity disorder. *Am J Psychiatry*, 2009, 166: 58-63.

Sheehan, D. V., Sheehan, K. H., Shytle, R. D., Janavs, J., Bannon, Y., Rogers, J. E., Milo, K. M., Stock, S. L. and Wilkinson, B. Reliability and validity of the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID). *J Clin Psychiatry*, 2010, 71: 313-26.

Silk, J. S., Vanderbilt-Adriance, E., Shaw, D. S., Forbes, E. E., Whalen, D. J., Ryan, N. D. and Dahl, R. E. Resilience among children and adolescents at risk for depression: Mediation and moderation across social and neurobiological contexts. *Dev Psychopathol*, 2007, 19: 841-65.

Society, A. T. Standardization of Spirometry, 1994 Update. *Am J Respir Crit Care Med*, 1995, 152: 371-75.

Sowell, E. R., Thompson, P. M. and Toga, A. W. Mapping changes in the human cortex throughout the span of life. *Neuroscientist*, 2004, 10: 372-92.

Spruyt, K., Raubuck, D. L., Grogan, K., Gozal, D. and Stein, M. A. Variable sleep schedules and outcomes in children with psychopathological problems: preliminary observations. *Nat Sci Sleep*, 2012, 4: 9-17.

Stadelmann, K., Latshang, T. D., Lo Cascio, C. M., Tesler, N., Stoewhas, A. C., Kohler, M., Bloch, K. E., Huber, R. and Achermann, P. Quantitative changes in the sleep EEG at moderate altitude (1630 m and 2590 m). *PLoS One*, 2013, 8: e76945.

Stanley, N. The future of sleep staging. *Hum Psychopharm Clin*, 1996, 11: 253-56.

Steiger, A. and Kimura, M. Wake and sleep EEG provide biomarkers in depression. *J Psychiatr Res*, 2010, 44: 242-52.

Stephan, K. E., Baldeweg, T. and Friston, K. J. Synaptic plasticity and dysconnection in schizophrenia. *Biol Psychiatry*, 2006, 59: 929-39.

Steriade, M. Corticothalamic resonance, states of vigilance and mentation. *Neuroscience*, 2000, 101: 243-76.

Steriade, M. Grouping of brain rhythms in corticothalamic systems. *Neuroscience*, 2006, 137: 1087-106.

Steriade, M., Contreras, D., Curro Dossi, R. and Nunez, A. The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J Neurosci*, 1993a, 13: 3284-99.

Steriade, M., Mccormick, D. A. and Sejnowski, T. J. Thalamocortical Oscillations in the Sleeping and Aroused Brain. *Science*, 1993b, 262: 679-85.

Steriade, M., Nunez, A. and Amzica, F. Intracellular Analysis of Relations between the Slow (Less-Than-1 Hz) Neocortical Oscillation and Other Sleep Rhythms of the Electroencephalogram. *J Neurosci*, 1993c, 13: 3266-83.

Steriade, M., Nunez, A. and Amzica, F. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci*, 1993d, 13: 3252-65.

Steriade, M. and Timofeev, I. Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron*, 2003, 37: 563-76.

Steriade, M., Timofeev, I. and Grenier, F. Natural waking and sleep states: a view from inside neocortical neurons. *J Neurophysiol*, 2001, 85: 1969-85.

Stickgold, R. Parsing the role of sleep in memory processing. *Curr Opin Neurobiol*, 2013, 23: 847-53.

Stowhas, A. C., Latshang, T. D., Lo Cascio, C. M.*, et al.* Effects of acute exposure to moderate altitude on vascular function, metabolism and systemic inflammation. *PLoS One*, 2013, 8: e70081.

Sturman, D. A. and Moghaddam, B. The neurobiology of adolescence: changes in brain architecture, functional dynamics, and behavioral tendencies. *Neurosci Biobehav Rev*, 2011, 35: 1704-12.

Stuss, D. T. Functions of the frontal lobes: relation to executive functions. *J Int Neuropsychol Soc*, 2011, 17: 759-65.

Tandon, R., Shipley, J. E., Taylor, S., Greden, J. F., Eiser, A., Dequardo, J. and Goodson, J. Electroencephalographic sleep abnormalities in schizophrenia. Relationship to positive/negative symptoms and prior neuroleptic treatment. *Arch Gen Psychiatry*, 1992, 49: 185-94.

Tarokh, L., Carskadon, M. A. and Achermann, P. Trait-like characteristics of the sleep EEG across adolescent development. *J Neurosci*, 2011, 31: 6371-8.

Tau, G. Z. and Peterson, B. S. Normal development of brain circuits. *Neuropsychopharmacology*, 2010, 35: 147-68.

Taylor Tavares, J. V., Clark, L., Furey, M. L., Williams, G. B., Sahakian, B. J. and Drevets, W. C. Neural basis of abnormal response to negative feedback in unmedicated mood disorders. *Neuroimage*, 2008, 42: 1118-26.

Tesler, N., Gerstenberg, M. and Huber, R. Developmental changes in sleep and their relationships to psychiatric illnesses. *Curr Opin Psychiatry*, 2013, 26: 572-9.

Teyler, T. J., Cavus, I. and Coussens, C. Synaptic plasticity in the hippocampal slice: functional consequences. *J Neurosci Methods*, 1995, 59: 11-7.

Thapar, A., Collishaw, S., Pine, D. S. and Thapar, A. K. Depression in adolescence. *Lancet*, 2012, 379: 1056-67.

Thapar, A. and Rice, F. Twin studies in pediatric depression. *Child Adolesc Psychiatr Clin N Am*, 2006, 15: 869-81, viii.

Thompson, P. M., Vidal, C., Giedd, J. N., Gochman, P., Blumenthal, J., Nicolson, R., Toga, A. W. and Rapoport, J. L. Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. *Proc Natl Acad Sci U S A*, 2001, 98: 11650-5.

Thurnheer, R., Xie, X. and Bloch, K. E. Accuracy of nasal cannula pressure recordings for assessment of ventilation during sleep. *Am J Respir Crit Care Med*, 2001, 164: 1914-9.

Timofeev, I., Grenier, F. and Steriade, M. Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. *Proc Natl Acad Sci U S A*, 2001, 98: 1924-9.

Tobin, M. J., Mador, M. J., Guenther, S. M., Lodato, R. F. and Sackner, M. A. Variability of Resting Respiratory Drive and Timing in Healthy-Subjects. *J Appl Physiol*, 1988, 65: 309-17.

Tobler, I. [Why do we sleep? Contributions from animal research]. *Ther Umsch*, 2000, 57: 417-20.

Todd, R. D., Neuman, R., Geller, B., Fox, L. W. and Hickok, J. Genetic studies of affective disorders: should we be starting with childhood onset probands? *J Am Acad Child Adolesc Psychiatry*, 1993, 32: 1164-71.

Tombaugh, T. N. Trail Making Test A and B: normative data stratified by age and education. *Arch Clin Neuropsychol*, 2004, 19: 203-14.

Tomitaka, S., Tomitaka, M., Tolliver, B. K. and Sharp, F. R. Bilateral blockade of NMDA receptors in anterior thalamus by dizocilpine (MK-801) injures pyramidal neurons in rat retrosplenial cortex. *Eur J Neurosci*, 2000, 12: 1420-30.

Tononi, G. and Cirelli, C. Sleep and synaptic homeostasis: a hypothesis. *Brain Res Bull*, 2003, 62: 143-50.

Tononi, G. and Cirelli, C. Sleep function and synaptic homeostasis. *Sleep Med Rev*, 2006, 10: 49-62.

Tononi, G. and Cirelli, C. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron*, 2014, 81: 12-34.

Uldry, C. and Fitting, J. W. Maximal Values of Sniff Nasal Inspiratory Pressure in Healthy-Subjects. *Thorax*, 1995, 50: 371-75.

Urner, M., Tornic, J. and Bloch, K. E. Sleep patterns in high school and university students: a longitudinal study. *Chronobiol Int*, 2009, 26: 1222-34.

Urrila, A. S., Karlsson, L., Kiviruusu, O., Pelkonen, M., Strandholm, T. and Marttunen, M. Sleep complaints among adolescent outpatients with major depressive disorder. *Sleep Med*, 2012, 13: 816-23.

Van Os, J., Linscott, R. J., Myin-Germeys, I., Delespaul, P. and Krabbendam, L. A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness-persistence-impairment model of psychotic disorder. *Psychol Med*, 2009, 39: 179-95.

Van Stegeren, A. H., Wolf, O. T., Everaerd, W., Scheltens, P., Barkhof, F. and Rombouts, S. A. Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. *Neurobiol Learn Mem*, 2007, 87: 57-66.

Virues-Ortega, J., Buela-Casal, G., Garrido, E. and Alcazar, B. Neuropsychological functioning associated with high-altitude exposure. *Neuropsychol Rev*, 2004, 14: 197-224.

Vyazovskiy, V. V. and Harris, K. D. Sleep and the single neuron: the role of global slow oscillations in individual cell rest. *Nat Rev Neurosci*, 2013, 14: 443-51.

Vyazovskiy, V. V., Olcese, U., Lazimy, Y. M., Faraguna, U., Esser, S. K., Williams, J. C., Cirelli, C. and Tononi, G. Cortical firing and sleep homeostasis. *Neuron*, 2009, 63: 865-78.

Vyazovskiy, V. V., Riedner, B. A., Cirelli, C. and Tononi, G. Sleep homeostasis and cortical synchronization: II. A local field potential study of sleep slow waves in the rat. *Sleep*, 2007, 30: 1631-42.

Wagner, U. and Born, J. Memory consolidation during sleep: Interactive effects of sleep stages and HPA regulation. *Stress*, 2008, 11: 28-41.

Wamsley, E. J., Tucker, M. A., Shinn, A. K., Ono, K. E., Mckinley, S. K., Ely, A. V., Goff, D. C., Stickgold, R. and Manoach, D. S. Reduced sleep spindles and spindle coherence in schizophrenia: mechanisms of impaired memory consolidation? *Biol Psychiatry*, 2012, 71: 154-61.

Wechsler, D. Wechsler Adult Intelligence Scale-Revised (WAIS-R). *San Antonio, TX: The Psychological Corporation*, 1981

White, T., Ho, B. C., Ward, J., O'leary, D. and Andreasen, N. C. Neuropsychological performance in first-episode adolescents with schizophrenia: a comparison with first-episode adults and adolescent control subjects. *Biol Psychiatry*, 2006, 60: 463-71.

Whitlock, J. R., Heynen, A. J., Shuler, M. G. and Bear, M. F. Learning induces long-term potentiation in the hippocampus. *Science*, 2006, 313: 1093-97.

Wiesel, T. N. and Hubel, D. H. Effects of Visual Deprivation on Morphology and Physiology of Cells in the Cats Lateral Geniculate Body. *J Neurophysiol*, 1963, 26: 978-93.

Wilhelm, I., Kurth, S., Ringli, M., Mouthon, A. L., Buchmann, A., Geiger, A., Jenni, O. G. and Huber, R. Sleep slow-wave activity reveals developmental changes in experience-dependent plasticity. *J Neurosci*, 2014, 34: 12568-75.

Yang, C. and Winkelman, J. W. Clinical significance of sleep EEG abnormalities in chronic schizophrenia. *Schizophr Res*, 2006, 82: 251-60.

Yoon, S. Y., Jain, U. and Shapiro, C. Sleep in attention-deficit/hyperactivity disorder in children and adults: past, present, and future. *Sleep Med Rev*, 2012, 16: 371-88.

Young, W., Knowles, J. B., Maclean, A. W., Boag, L. and Mcconville, B. J. The sleep of childhood depressives: comparison with age-matched controls. *Biol Psychiatry*, 1982, 17: 1163-8.

Zuo, Y., Lin, A., Chang, P. and Gan, W. B. Development of long-term dendritic spine stability in diverse regions of cerebral cortex. *Neuron*, 2005, 46: 181-89.

5

Curriculum Vitae

Noemi Agneta Tesler

Born 03.04.1985 in Timisoara, Romania

Academic Education:

06.10 – 11.14 **University of Zurich/ETH**
- PhD studies in Neuropsychology at the **University of Zurich** and in Neuroscience at the **Neuroscience Center Zurich** (also short collaborative stays at the **VU Amsterdam - Neuroscience Campus**)

10.08 – 11.09 **Stanford University Medical School –** Institute for Psychiatry and Behavioral Sciences, Palo Alto, CA, USA
- Academic exchange year / internship
- Research and writing of the MA- thesis

10.04 – 01.10 **Technical University of Dresden**, Faculty of Science, Dresden, Germany
- Majors: neuro- and clinical psychology
- Minors: work and organizational psychology, law

09.92 – 06.04 **German school**/highschool "Nikolaus Lenau" in **Timisoara**, Romania

Professional Experience:

since 09.10 Research Associate at the **University Children's Hospital Zurich** and **Child and Adolescent Psychiatry, University of Zurich,** Switzerland

06.10 – 09.10 Research Assistant at the Department of Pneumology and Sleep research at the **University Hospital Zurich** and the **Clinics Davos Klosters,** Switzerland

10.08 – 11.09 Research Assistant at the Institute for Psychiatry and Behavioral Sciences, **Stanford University Medical School**, United States

10.07 – 10.08 Research Assistant at the Institute for Clinical Psychology and Psychotherapy, **University of Dresden**, Germany

08.07 – 10.07 Internship in Human Resources at „Health Care Communication" in **Vienna**, Austria

06.06 – 06.08 Employee at the administration department of the „**Palucca University Dresden**", Dresden, Germany

07.04 – 08.04 Translator for the company „Conrad – Electronic", Germany (German, English, Romanian)

Scholarships:

06.10 – 08.13	Erasmus Mundus European Neuroscience Campus fellowship for a 3 year Joint Phd Programme at the University of Zurich/ETH (Neuroscience Center) and the VU Amsterdam (Neuroscience Campus)
08.13	Novartis Biotechnology Leadership Camp 2013, Novartis International Headquarters, Basel, Switzerland
09.12	Travel grant from the Swiss Society for Sleep Research and Chronobiology (SSSSC) for the European Sleep Research Society conference in Paris 2012
01.07 – 12.09	Grantee of the German college association in Timisoara, Romania
09.08 – 11.09	Grantee of the Technical University of Dresden and the Institute for Clinical Psychology and Psychotherapy Dresden
10.08 – 11.09	Grantee of the Institute for Psychiatry and Behavioral Sciences, Stanford Medical School
04.03	DaimlerChrysler fellow for a scholar exchange in Stuttgart, Germany

Language competencies:

Hungarian – native
Romanian – native
German – native
English – C2
French – B2
Spanish – A2
Italian – A2

Awards:

- **Best poster award: N Tesler**, M Gerstenberg, M Franscini, OG Jenni, S Walitza, R Huber (2014): „Reduced sleep spindle density in early onset schizophrenia“ in collaboration with the Child and Adolescent Psychiatry Zurich (poster presented at the FZK meeting 2014, Au)

- **Best poster award: N Tesler**, M Gerstenberg, A Preiss, M Franscini, OG Jenni, S Walitza, R Huber (2013): „EEG sleep slow wave activity in adolescents with Depression“ in collaboration with the Child and Adolescent Psychiatry Zurich (poster presented at the SSSSC meeting 2013, Aarau)

- **Second best presentation award: N Tesler**, M Gerstenberg, A Preiss, M Franscini, OG Jenni, S Walitza, R Huber (2013): „EEG sleep slow wave activity in adolescents with Depression“ in collaboration with the Child and Adolescent Psychiatry Zurich (presented at the Erasmus Mundus meeting in Bordeaux 2013)

Articles in peer-reviewed journals:

- **N Tesler**, M Gerstenberg, R Huber (2013): „Developmental changes in sleep and their relationships to psychiatric illnesses“ - review article (Current Opinion in Psychiatry, accepted)
- **N Tesler**, M Gerstenberg, M Franscini, A Preiss, OG Jenni, S Walitza, R Huber (2014): „Reduced sleep spindle activity in early onset schizophrenia“ (submitted)
- **N Tesler**, M Gerstenberg, M Franscini, OG Jenni, S Walitza, R Huber (2014): „Increased frontal slow wave activity in adolescents with Major Depression and their unaffected siblings“ (submitted)
- C Lustenberger, Mouthon AL, **N Tesler**, Kurth S, Ringli M, Pugin F, Jenni OG, R Huber: „Individual Slow Wave Activity Trajectories as a Marker for Brain Development“ (in preparation)
- **N Tesler**, TD Latshang, CM Lo Cascio, K Stadelmann, AC Stoewhas, M Kohler, KE Bloch, P Achermann, R Huber (2013): „Ascent to moderate altitude impairs overnight memory improvement“ (Physiology and Behavior, accepted)
- A Aepli, S Kurth, **N Tesler**, OG Jenni, R Huber (2014): „Altered sleep behaviour and sleep electroencephalographic slow wave activity in regularly caffeine consuming children and adolescents“ (in preparation)
- TD Latshang, CM Lo Cascio, AC Stoewhas, M Grimm, K Stadelmann, **N Tesler**, P Achermann, R Huber, M Kohler, KE Bloch (2013): „Are nocturnal breathing, sleep and cognitive performance impaired at moderate altitude (1630 to 2590m)“ (Sleep, accepted)
- Stöwhas AC, Latshang TD, Lo Cascio CM, Lautwein S, Stadelmann K, **Tesler N**, Ayers L, Berneis K, Gerber PA, Huber R, Achermann P, Bloch KE, Kohler M (2013): „Effects of acute exposure to moderate altitude on vascular function, metabolism and systemic inflammation“ (PLoS One, accepted)
- K Stadelmann, TD Latshang, L Tarokh, CM Lo Cascio, **N Tesler**, AC Stoewhas, M Kohler, KE Bloch, R Huber, P Achermann (2013): „Quantitative changes in the sleep EEG at moderate altitude (1630 m and 2590 m)“ (PLoS One, accepted)
- K Stadelmann, TD Latshang, CM Lo Cascio, **N Tesler**, AC Stoewhas, M Kohler, KE Bloch, R Huber, P Achermann (2014): „Sleep respiratory disturbances and arousals at moderate altitude have overlapping electroencephalogram spectral signatures“ (Journal of Sleep Research, accepted)
- Ayers L, Stöwhas AC, Ferry B, Latshang TD, Lo Cascio CM, Sadler R, Stadelmann K, **Tesler N**, Huber R, Achermann P, Bloch KE, Kohler M (2013): „Effects of acute exposure to moderate altitude on vascular function, metabolism and systemic inflammation“ (European Journal of Applied Physiology, accepted)

- Weise, S, Ong, J, **Tesler NA**, Kim, S, Roth WT (2013): „Worried sleep: 24h monitoring in high and low worriers“ (Biological Psychology, accepted)

Conference contributions:

Presentations

- **N Tesler**, M Gerstenberg, M Franscini, OG Jenni, S Walitza, R Huber (2014): „Reduced sleep spindle density in early onset schizophrenia“ (presented at the First Burghölzli Psychiatry Meeting, Zurich)
- **N Tesler**, M Gerstenberg, A Preiss, M Franscini, OG Jenni, S Walitza, R Huber (2013): „EEG sleep slow wave activity in adolescents with Depression “ (presented at the Erasmus Mundus ENC annual meeting 2013, Bordeaux)
- **N Tesler**, M Gerstenberg, A Preiss, M Franscini, OG Jenni, S Walitza, R Huber (2013): „EEG sleep slow wave activity in adolescents with Depression“ (presented at the Young Scientist Symposium at the SGKJPP meeting 2012, Zurich)

Posters

- **N Tesler**, M Gerstenberg, M Franscini, OG Jenni, S Walitza, R Huber (2014): „Reduced sleep spindle density in early onset schizophrenia“ (poster presented at the FZK meeting 2014, Au, Switzerland)

- **N Tesler**, M Gerstenberg, M Franscini, A Preiss, OG Jenni, S Walitza, R Huber (2014): „Increased frontal slow wave activity in adolescents with Major Depression and their unaffected siblings“ (poster presented at the ESRS 2014, Talinn, Estonia)

- **N Tesler**, M Gerstenberg, A Preiss, M Franscini, OG Jenni, S Walitza, R Huber (2013): „EEG sleep slow wave activity in adolescents with Depression“ in collaboration with the Child and Adolescent Psychiatry Zurich (poster presented at the SSSSC meeting 2013, Aarau, Switzerland)

- **N Tesler**, M Gerstenberg, A Preiss, M Franscini, OG Jenni, S Walitza, R Huber (2013): „EEG sleep slow wave activity in adolescents with Depression“ in collaboration with the Child and Adolescent Psychiatry Zurich (poster presented at the ENCODS meeting 2013, Bordeaux, France)

- **N Tesler**, M Gerstenberg, A Preiss, M Franscini, OG Jenni, S Walitza, R Huber (2013): „Topography of sleep sow wave activity in adolescents with affective disorders“ in collaboration with the Child and Adolescent Psychiatry Zurich (poster presented at the SGKJPP meeting 2013, Zurich, Switzerland)

- **N Tesler**, TD Latshang, CM Lo Cascio, K Stadelmann, AC Stoewhas, M Kohler, KE Bloch, P Achermann, R Huber (2012): „Exposure to moderate altitude affects sleep slow waves and post sleep performance improvement“ in collaboration with the University Hospital Zurich (poster presented at the ESRS 2012, Paris, France)

- **N Tesler**, TD Latshang, Y Nussbaumer-Ochsner, CM Lo Cascio, K Stadelmann, AC Stoewhas, M Kohler, KE Bloch, P Achermann , R Huber (2011): „Alterations in slow wave sleep characteristics after acute exposure to moderate altitude in healthy controls and patients with obstructive sleep apnea“ in collaboration with the University Hospital Zurich (poster presented at the FENS 2012, Barcelona, Spain)

- **N Tesler**, TD Latshang, Y Nussbaumer-Ochsner, CM Lo Cascio, K Stadelmann, AC Stoewhas, M Kohler, KE Bloch, P Achermann , R Huber (2011): „Alterations in slow wave sleep characteristics after acute exposure to moderate altitude in controls and patients with obstructive sleep apnea “ in collaboration with the University Hospital Zurich (poster presented at the SSSSC meeting 2012, Zurich, Switzerland)

- **N Tesler**, TD Latshang, Y Nussbaumer-Ochsner, CM Lo Cascio, K Stadelmann, AC Stoewhas, M Kohler, KE Bloch, P Achermann , R Huber (2011): „Alterations in slow wave sleep characteristics after acute exposure to moderate altitude“ in collaboration with the University Hospital Zurich (poster presented at the SSN meeting 2012, Zurich, Switzerland)

- Kim, S., Wollburg, E., **Tesler, N.**, Roth W. T. (2008): “Effects of Capnometer-Assissted Breathing Therapy on Sleep in Panic Disorder Patients” in collaboration with the Stanford University Medical School and the Veterans Hospital – Palo Alto, CA, USA (poster presented 2009 at the conferences of the „American Psychological Association“ in Toronto and the „American Behavioral and Cognitive Therapies“ in New York, USA)

- Doberenz, S., Sunyoung, K., **Tesler, N.**, Roth W. T.(2009): “Worried sleep: 24-hr monitoring in high and low worriers” in collaboration with the Stanford University Medical School and the Veterans Hospital – Palo Alto, CA, USA (oral presentation, presented in May 2010 at the „Anxiety Disorders Association of America“ in Baltimore, USA)

- Doberenz, S., Roth, W. T., **Tesler N.**, Kim, S. (2009): „Worrying and its effects on electrodermal and cardiac autonomic activity“ in collaboration with the Stanford University Medical School and the Veterans Hospital – Palo Alto, CA, USA (poster presented 2010 at the „Anxiety Disorders Association of America“, USA)

6

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